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CORRECTION

Delete the sentence "Thirty to 60 cc. of olive oil or mineral oil should be left in the stomach" which appears on page 774 (lines 18 and 19) of the November, 1954, number of the PEDIATRIC CLINICS OF NORTH AMERICA.

FOREWORD

EDWARD B. SHAW, M.D.

This Symposium on the Unusual Infections of Childhood is planned to include a variety of infectious diseases which the average physician dealing with children may encounter but rarely—except perhaps in certain areas of the globe—and to serve him as a ready reference and guide for their diagnosis and care.

There was some uncertainty about the infections which should be included, and the list might well be greatly extended. In medicine, as in everything else, nothing is constant but change. One hundred years ago smallpox could scarcely have been regarded as unusual, but at that same time poliomyelitis was a rare disease. Many of the infections which were common two or three decades ago have now, because of specific methods of protection, become so uncommon as almost to justify their inclusion in this discussion.

At no time in the world's history has medical knowledge been so rapidly expanded as in the last few years. Discoveries of new infectious agents and methods for specific diagnosis and definitive treatment have been so rapidly acquired as to tax the resources of the physician to keep himself abreast.

No matter how well informed the clinician may be, he will sometimes encounter illnesses which are foreign to his experience and in which he will require immediate and adequate information for his successful management of the patient.

The literature of current medical journals suffices to add that which is new to our information, but much that is true is not new and that which is new is not always true. Textbooks necessarily lag behind the advances of knowledge, if only to await complete evaluation of new concepts. Furthermore, textbooks sometimes perpetuate by repetition ideas which are little better than medical folklore.

Both textbooks and current journals frequently fail to be sufficiently explicit to guide the physician in unfamiliar territory. I remember that

when I was in medical school a text of impeccable authority recommended the use of the milk of thyroidectomized goats as a treatment for exophthalmic goiter—leaving me wistfully to wonder how this was to be procured, how much to give, and what effect was to be expected!

The practice of medicine is hardly the occupation for the inept or the amateur, but expert chefs do not despise the cookbook, nor do lawyers fail to consult established opinion. This volume will have served its purpose if it provides an acceptable plan for medical management of these unusual conditions which the physician may follow until his own experience and resources provide better answers to his problems. Contributors have been encouraged to write their own opinions based upon their experience and knowledge and to be succinct and explicit. Each of the discussants is one who may safely speak with this authority.

It is sincerely hoped that this Symposium will contribute to the effectiveness of the clinician in some of the rare and difficult problems of practice with children.

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PASTEURELLA INFECTIONS

K. F. MEYER, M.D.

Several microbial agents that cause somewhat similar diseases in man now form the genus *Pasteurella*, so named in honor of Louis Pasteur, who discovered a species responsible for fowl cholera. The species most prominent in the frequency with which it has caused epidemics is *Pasteurella pestis*. Though morphologically and metabolically in no way related, the bacterium causing tularemia has been included with the *Pasteurella*, because this disease is occasionally mistaken for plague and some anatomic lesions of *Bacterium tularense* resemble those of *P. pestis*. The microbiologist will find a more appropriate place for the agent responsible for tularemia. The plague bacillus will probably be placed in a separate genus, *Yersinia*, and the pseudotuberculosis bacillus in the genus *Enterobacteriaceae*. The infections caused by organisms that now comprise the genus *Pasteurella* will be discussed.

PLAGUE

ETIOLOGY

In the tissues, *P. pestis* appears as a short, round coccoid or large ovoid, safety-pin-shaped, gram-negative, nonmotile bacillus. The typical bipolar staining is not so well seen in organisms cultured on artificial media. The organism is aerobic and grows well on media containing cystine and blood. Under favorable conditions it remains viable for months, even years; in pus and sputum for one to two weeks; in rat-flea fecal pellets for months; and when frozen, almost indefinitely. It is inactivated at 55° C. or by a 0.5 per cent solution of phenol in fifteen minutes. The plague bacillus has a highly active immunogenic antigen (fraction I) and elaborates a highly potent toxin.

From the George Williams Hooper Foundation, University of California, San Francisco.

INCIDENCE

The incidence of plague has decreased in recent years, but there are still active centers throughout the world. After 448 cases (286 deaths) the United States saw the last of the plague of murine origin in 1924.[16] There were seventy-seven cases (fifty-four deaths) due directly or indirectly to wild rodents between 1908 and 1951. Although in 138 counties in fifteen states plague has been found among rodents and rat-caused plague has repeatedly appeared in the United States, it has not tended to spread to man and persist.[11] This is remarkable because commensal rats collected from many plague-infected localities have been heavily infested with the Oriental rat flea, an efficient plague vector. In wooded areas sparsely inhabited by man where heavy epizootics have occurred, human contact with the infective agent has seldom been established.

The age and sex incidence are little known. Opinions about the susceptibility of children vary from one extreme to the other, and until more extensive statistics are available it can only be said that children do contract plague and succumb to it.

SOURCES OF INFECTION

The basic infection chain is from rodent to rodent through the pulicine vector. The common sources of human infection are blood of infected rodents with terminal septicemia and sputum of patients with plague pneumonia. The route is the infective arthropod or airborne droplets. Skins, saliva or carcasses of wild rodents are infectious in exceptional cases. Infections may be contracted at autopsy of persons who succumbed to plague.

Urban or domestic plague, transmitted almost entirely from commensal rats, too often breaks out in densely populated, insanitary areas, can spread overland and has crossed oceans in ship cargoes. Rural plague, including campestral and sylvatic plague, the prevalent form in the United States, is transmitted among hunters, workers in woods, and children, through rodent bites or handling and perhaps infrequently through insect bites. It is primarily sporadic, bubonic plague, but has at times instituted pneumonic epidemics.

Primary pneumonic plague is often due to contact with patients who suffer from bubonic plague with secondary lung involvement. *Pasteurella pestis* is projected several feet from the face of the patient during coughing spells. *Failure to recognize and effectively treat bubonic plague may allow development of secondary pneumonia and subsequent rapid spread of the infection.*

PATHOLOGY

The findings in bubonic plague are (1) vesiculation of the flea bite wound; (2) involvement of the lymph nodes. Congestion, hemorrhage and edema surround the lymph nodes, especially those comprising the primary bubo. This perinodular infiltration and hemorrhagic extravasation are pathognomonic. (3) Toxic damage of the vascular and lymphatic endothelium, manifest in distention of veins, extravasation of blood and cutaneous and serosal petechiae; (4) congestion of subcutaneous capillaries, parenchymatous organs, meninges and brain; (5) hypertrophy of the spleen; (6) enlargement of the liver, engorgement, cloudy swelling or fatty degeneration and always softening; (7) congestion of the kidneys with cloudy swelling; (8) filling of the pericardial sac with straw-colored fluid; (9) dilatation of the right side of the heart, occasionally ecchymosis in the pericardium and endocardium; (10) coagulation or semifluidity of the blood. The organisms do not multiply in the blood extensively until the terminal stages. Death in all probability is due to the action of the toxin on the peripheral capillaries, causing hemoconcentration and shock.

In septicemic plague generalized congestion and hypertrophy of the spleen may be the only gross changes; careful search may reveal a lymph node with the characteristic perinodular reaction.

In primary plague pneumonia the following are characteristic: (1) slight hyperemia of the mucosa of the upper part of the respiratory tract; (2) congestion of the trachea and bronchi and filling with frothy, blood-stained exudate; (3) recent fibrinous pleural adhesions and hemorrhages beneath the visceral pleura, particularly over pneumonic areas; (4) concentrated pulmonary hemorrhagic extravasation in the form of congestion and edema or lobular pneumonic foci situated near the surface or within the lobes of the lungs and sometimes arranged along the bronchi; (5) involvement of lymph nodes. Bronchial nodes are usually enlarged, hyperemic and soft.

CLINICAL ASPECTS

The onset is sudden, and in almost all cases the picture is that of a severe infection. The abrupt onset may be preceded by malaise, headache, giddiness, apathy or restlessness, nausea, and pain in the limbs or the lumbar region. A feeling of chilliness is accompanied by rapid rise in temperature to 103° or 104° F.; the pulse rate increases and may become dicrotic or even intermittent early in the severe disease. Premonitory symptoms, onset and progress may vary with the number of *P. pestis* introduced by the vector or soiled into the skin abrasion or bite wound of a wild rodent. Ill defined fever, pain in the groin, armpit or neck, or a nodular swelling or bubo at the

seat of the pain, tender to pressure, characterize the acute benign bubonic form. It may last forty-eight hours, one week or two or three months. It is accompanied by suppuration and induration of the buboes, secondary anemia and debility.

In the severe *bubonic form* the symptoms develop rapidly; they offer nothing to distinguish the disease at the early stage unless the lymph nodes are enlarged. The fever may be severe, continued, remittent or irregular, the skin hot and dry, and the face flushed. Rapid pulse and respiration, intense thirst, nausea and vomiting and white coating of the tongue are common. Discomfort to intense pain directs the attention of the patient to the inflamed lymph node, which may remain small, hard and tense, but more frequently enlarges to the size of a walnut or goose egg and is embedded in a boggy edematous swelling.

Buboes occur in the inguinal region in about 70 per cent of the cases, particularly distal to Poupart's ligament; in the axillary region in about 20 per cent; and in the cervical region in about 10 per cent, particularly under the maxilla.

Unless treatment is started early, the disease progresses. There is much restlessness, overpowering fatigue and drowsiness; the patient looks worried or listless and apathetic. Death may occur between the second and the sixth days, usually from the effect of the toxin on the heart, leading to cardiac failure, or because of involvement of the lungs, or shock. The untreated patient's chances of recovery, as a rule tedious and protracted, are improved if he survives the first seven days. The buboes suppurate and discharge serous pus. The pulse may continue to be irregular, and as late as the twentieth day the patient's strength may fail and he may die. The common complications are pneumonia and septicemia. In rare instances bacilli localize in the meninges,[15] tonsils or intestines.

In *septic plague* peripheral buboes are absent, but buboes may be found in the iliac or lumbar region by deep palpation. Plague bacilli are easily revealed by blood smears. The patient is profoundly affected by the toxin. The rapidity with which he sickens is striking; death may occur between the first and third days. A pale countenance depicting intense anxiety, extreme prostration, temperature rarely above 103° F., hurried respiration and rapid pulse, involuntary evacuation, epistaxis and hematuria appear in rapid succession.

In *pneumonic plague* the illness begins with chills, general malaise, severe headache, nausea, vomiting and general pain, difficult and hurried breathing. Cough begins, and becomes more frequent; the sputum may at first be purely mucoid or mucopurulent, but soon contains specks or streaks of bright blood. The physical signs in the thorax are far fewer than one would expect on the basis of the autopsy

findings. On auscultation the signs of lobular pneumonia are present, but rarely is there appreciable dullness at the spots of crepitation, and the severity of the symptoms is entirely out of proportion to the physical signs. X-ray examinations, conducted for the first time in Madagascar, showed that pneumonic foci form early in the disease. Lung symptoms and cardiac distress increase, delirium supervenes, and the untreated patient becomes cyanotic and dies, usually on the second to fourth day. Besides being the most infectious, the pneumonic is the most fatal form.

DIAGNOSIS

Clinical Diagnosis

The illness of a boy bitten on the left thumb by a squirrel was diagnosed successively, while he was being studied at a hospital, as tularemia, meningitis, diphtheria, streptococcal septicemia and bronchopneumonia. The correct diagnosis was plague. A girl seven years old, bitten by a chipmunk, was hospitalized because of pain in the left arm and a temperature of 104° F. Surgical exploration of the axilla revealed edematous infiltration and a few enlarged lymph nodes. The diagnosis was made at autopsy, although the blood culture ante mortem yielded streptococci and *P. pestis*. In another case a typical inguinal bubo developed in a ten year old boy after he had been bitten by a wild rodent flea. Surgical treatment overtaxed the defense mechanism and hastened the fatal issue. The use of penicillin, instead of an antimicrobial drug effective against plague, in the treatment of another boy with an enlarged lymph node thought to be infected by staphylococci allowed the plague infection to run a fatal course.

Such things happen, even in areas where most of the inhabitants and certainly the physicians are aware of the existence of plague, when this infection is not considered. In retrospect it was realized that the clinical manifestations in these four cases were characteristic of plague. But the diagnosis must rest on the results of laboratory examinations except in well characterized cases during fully confirmed outbreaks or epidemics. Where plague is enzootic among wild rodents this infection must be considered by the physician in every suggestive case, and he should secure specimens for etiologic diagnosis—blood, fluid from the bubo, or sputum. Plague is reportable to public health officials by telephone, and they may be consulted about the proper handling and destination of diagnostic specimens.

Laboratory Diagnosis

Blood Culture. A blood sample (5 ml.) from the cubital vein is enriched in hormone-cystine broth, or 0.25 ml. is distributed on several

plain agar slants or plates to reveal septicemia and its degree. More than ten colonies indicates severe septicemia; fewer represent temporary showers of mild septicemia. Blood is infected early and again in the terminal stages, but the bacilli disappear under treatment and when active immunity develops. A positive culture does not always indicate a serious prognosis.

Blood Cell Counts and Smears. Smears may reveal plague bacilli and polymorphonuclear leukocytosis with a shift to the left (20,000 to 25,000).

Bubo Puncture. By puncture of an involved lymph node at the earliest stage of the infection and examination of the fluid, a bacteriologic diagnosis can be secured within a few minutes. The examiner and his assistant should wear rubber gloves and gauze mask. With an 18-gauge needle mounted on a well fitted 5- to 10-ml. syringe, gelatinous edema fluid is aspirated (the needle and syringe may be rinsed with saline solution if the fluid is scarce). The skin over the bubo is cleansed thoroughly and painted with tincture of iodine. After aspiration the puncture wound is disinfected with bichloride of mercury or absolute alcohol. Care must be exercised in expelling the few drops of fluid on a blood plate or agar slant and in making thin films on several slides, because bacilli may be vaporized and inhaled. The air-dried smear should be fixed with 95 per cent ethanol or, preferably, with absolute methanol.

Sputum Examination. Primary pneumonic plague can be diagnosed by examination of smears of sputum, in which plague bacilli are abundant. Culture on blood or gentian violet (1:70,000) plates or animal tests yield results comparatively rapidly.

Serodiagnostic Procedures. Adequate treatment frequently makes it impossible to obtain bacteriologic proof of plague by bubo puncture or blood culture. Serologic tests, in particular the hemagglutination test with fraction I and the agglutination test executed by experienced laboratory workers, sometimes establish the diagnosis in retrospect.[3]

Autopsy Material. In the United States every state authorizes the physician or health officer to demand autopsy when plague is suspected. If complete autopsy is impossible, the bubo, bone marrow or pieces of spleen, liver and lung should be excised. Workers not familiar with *P. pestis* should refer the specimens to laboratory experts.

TREATMENT

Outstanding success in the specific treatment of all forms of plague has been attained with streptomycin and some other antimicrobials. Administration of sulfonamides is now recommended primarily for the abortive treatment of contacts of patients with secondary lung involve-

ment and under special conditions—lack of effective antimicrobials, laboratory or postmortem accidents, infection on a ship or in a port hitherto free from plague. The standard recommendation by the World Health Organization Expert Committee on Plague in 1952 was that daily doses of 3 gm. of a suitable sulfonamide (sulfadiazine) be administered for six days for prevention of plague. Streptomycin is highly bactericidal to *P. pestis*. The cure rate in human bubonic, septicemic and pneumonic plague has ranged from 90 to 100 per cent. Even pneumonic plague, if treated during the first twenty-four hours, may be cured. According to Girard,[6] a child with pneumonic plague recovered when given 11.5 gm. of streptomycin. Detailed records of treatment of plague in children with streptomycin are few, but the observations on adults recommend administration of 1 gm. daily for six days, given in 125-mg. doses at three hour intervals. Since in experimental work[13] drug-resistant strains have developed, Aureomycin, oxytetracycline (Terramycin) or chloramphenicol should replace streptomycin on the third day of treatment. After subcutaneous administration of 3 to 4 gm. of streptomycin, oral administration of Aureomycin or oxytetracycline succeeded in the few cases in which it was used. Aureomycin, in a total dose of 2.5 to 7.5 gm. for adults, given in individual doses of 250 mg. every two hours, cured one septicemic and eleven bubonic infections.[20]

Apart from precautions against spread, the following special considerations must be given to plague patients. Strict rest in bed during the acute illness is essential because exertion aggravates the condition and in particular may promote secondary lung involvement. Nourishing, adequately light diet and ample fluid intake should be provided. Supportive treatment is not essential; cardiac drugs, such as Coramine, have been used. Adrenocortical preparations are contraindicated. During the acute stage manipulation of the buboes should be absolutely avoided. Application of ichthyol belladonna ointment spread on gauze pads alleviates the pain. If pus forms, the buboes should be incised, but only when fluctuation has become general. The gauze strips draining the resulting wounds should be changed as seldom as possible.

On the patient's arrival at the hospital his clothing should be disinfected and disinfested of fleas in a steam sterilizer. Anyone who comes in contact with him must wear gloves, a special cotton gown and particularly a face mask made of eight to twelve layers of gauze covered by a deflection mask. The physician should make no examination without wearing goggles when pneumonic plague is suspected. The extreme infectivity of droplets and droplet nuclei should be kept in mind. The air in the rooms in which patients with pneumonic plague are housed should be kept dry.

Considering control in general, prophylactic inoculations are merely adjuncts to measures which simultaneously destroy both fleas and

rodents. The present state of plague in the United States does not warrant vaccination of children who may collide with *P. pestis* in rural areas. Finally, so long as the disease is diagnosed and treated and control measures are applied, there is no need to become alarmed and hypothesize that the Black Death may march again throughout the world. Plague once had a case fatality rate of 60 to 90 per cent, in the pneumonic form of 100 per cent, but even in India this has been reduced to 5 per cent.

TULAREMIA

During studies on campestral plague, G. W. McCoy discovered, among ground squirrels, a disease characterized by lesions similar to those of plague. It was found in rodents shot or found dead in Tulare, California, and the causative agent was named *Bacterium tularense*. In 1914 Wherry and Lamb recognized the first infection in man and the hare as an important source. Francis in 1919 and 1920, in investigations of rabbit fever in Utah, identified the deer fly as an important vector. Shortly thereafter investigators from the United States Public Health Service found ticks to be important reservoirs. Since then water-borne and murine outbreaks have assumed importance in Russia and the United States.[18]

ETIOLOGY

Bacterium tularense is a gram-negative, nonmotile, unencapsulated, highly polymorphous microorganism with obligate requirements for cystine. The morphologic units, ranging from less than 0.3 micron to 3 microns in diameter, comprise coccoid globi, globules, bacillary forms, delicate filaments and minute, filtrable minimal reproductive elements.

INCIDENCE

Tularemia has been reported from all parts of the United States except Vermont; some areas are much more heavily infected than others. It occurs in Alaska, Canada, Japan, eastern, northern and central Europe, Turkey, Asia Minor and North Africa. Since 1877 it has spread from western Siberia in waves through central Europe. It is likely to reappear in endemic areas in which the steppelike climate and vegetation favor a large rodent reservoir. Up to 1942 the mortality rate in the 15,525 cases in the United States was 6.9 per cent. In California over a period of twenty-five years the total number of cases was 268 (eight deaths).[21] The average per annum was eleven, with an occasional rise

to sixteen, but since 1947 it has dropped to six cases. The areas of highest incidence were those in which human contact with wild rabbits was greatest.

Age and sex distribution is determined by frequency of exposure, since neither directly influences susceptibility to tularemia. Reports on the incidence in children vary; some state that it is low, and others consider it a pediatric problem.[9] The attack rate in California for the age group 0 to nine years was 1.0, and for the group ten to nineteen years old, 2.5 per 100,000.[21] In Arkansas in a ten year period, 110 (14 per cent) of the 704 cases occurred in preschool and school children.

SOURCES

According to Parker, "no other infection of animals communicable to man can be acquired from sources so numerous and so diverse." Burroughs and his colleagues[2] list forty-eight species of vertebrate hosts, including the principal hosts—the cotton tails, jack rabbits and snowshoe rabbits. Ticks (*Haemaphysalis* and *Dermacentor*) are chiefly responsible for maintaining the infection among these hosts. The bionomics and ecology of arthropods and rodents vary from state to state, from country to country, forming a kaleidoscopic epidemiologic picture of great potentiality known only in its broader outlines.

Bacterium tularense depends on no single portal of entry; it may enter through (1) wounds or even the unbroken skin, usually during skinning and dressing of game. Human infections have not been traced to domestic rabbits, but such have been found infected. (2) Bites of arthropods, principally ticks and deer flies; (3) bites of animals—cats, dogs, opossums, squirrels, rats, field mice, hogs; (4) contact with sheep, in particular with their ticks and the fecal droplets in the wool; (5) air, by inhalation of dust contaminated with infected mouse feces in homes, trenches or during grain threshing; (6) food, in inadequately cooked hares or rabbits; (7) ingestion of water from tularemia-contaminated streams and well water.[18] Despite the frequency of tularemic pneumonia, easy recoverability of *Bact. tularense* by nasal and pharyngeal swab and the excretion by patients of pus or discharges quite infectious for animals, human-to-human infection has not been recorded.

PATHOLOGY

Tissues most frequently affected are skin, conjunctiva, regional or remote lymph nodes, lungs, spleen, liver and large serous membranes. The fundamental lesions in the acute and subacute stages are those of focalized necrosis.[10]

CLINICAL CHARACTERISTICS

Francis[5] originally described four clinical types—ulceroglandular, oculoglandular, glandular, and typhoidal; the pharyngotonsillar type has been added since. In the United States tularemia with primary lesions is found more frequently than the generalized type.

Clinical manifestations begin one to fifteen days after exposure; the average is reported to be from three to 4.6 days. The general symptoms, which may develop suddenly, are severe headache, vomiting, chills and fever with an initial rise in temperature above 104° F., accompanied by aching, weakness, prostration, occasional sweats and loss of weight. When the fever is severe, prostration is extreme and drenching sweats are frequent. The temperature does not follow a characteristic pattern; it may be relapsing, undulating, remittent or continuous. In the typhoidal type of infection an initial rise, remission and secondary rise are common. The febrile period may last ten to fifteen days; it usually runs a course of three to four weeks in the severe infections.

Eruptions of the skin—papular exanthema, pustules and petechiae—may appear at any stage in any form of tularemia. The pulse is only slightly accelerated; during convalescence, tachycardias are annoying. The spleen is somewhat enlarged in about a quarter of the cases. Slight to moderate polymorphonuclear leukocytosis (12,000 to 15,000) and mild secondary anemia are common. The sedimentation rate in patients with severe local lesions may be slightly accelerated. The output of urine is diminished, and albuminuria may be present temporarily.

The acute untreated disease may last for two weeks to fifteen months. The prognosis for survival is good in the absence of septicemia or pulmonary involvement. Most deaths occur between the eleventh and the eighteenth days of disease. In one series four (8 per cent) of forty-eight children with tularemia died.[9]

Cutanoglandular Type

Primary lesions usually develop at contaminated areas before buboes become apparent. From the uncharacteristic, painful, necrotizing papular state they enlarge to indolent ulcers with raised edges, vertical walls and ragged necrotic floors. The fingers, hands and arms are the usual sites, but the ulcers may arise on any part of the skin.

Oculoglandular Type

The primary localization is usually in the conjunctival sac and unilateral. Itching, photophobia and pain are subjective symptoms; they are accompanied by lacrimation and swelling of the preauricular, parotid, submaxillary and cervical lymph nodes. The eyelids are swollen, and

the chemotic, deep red, inflamed conjunctiva is studded with small, discrete, yellow nodules; punched-out ulcers follow the breakdown of the necrotic nodules. A mucopurulent discharge accompanies the conjunctivitis for three to five weeks after the swelling has receded. The usually benign tularemic conjunctivitis may become generalized and fatal.

Oral Glandular Type

Ingestion of insufficiently cooked infected meat or of contaminated water may cause lesions resembling those of follicular tonsillitis, Vincent's angina or even diphtheria. There are ulcers on the roof of the mouth, pharynx and nasopharynx and shaggy necrotic exudate, and the submaxillary and anterior cervical lymph nodes are enlarged. The lesions are usually benign and heal rapidly.

Thoracic and Abdominal Types

The bronchopneumonic changes frequently observed in the roentgenogram in adult patients with tularemia are not seen in children. The available reports of autopsy of children who succumbed to tularemic septicemia are singularly devoid of specific findings; they record that examination of the tongue, trachea, lungs, spleen, liver and other organs revealed tularemic involvement. By contrast, the descriptions of extensive ulceration of the jejunum, ileum and ileocecal area in fatal abdominal tularemia amply attest that the intestines are one primary portal of entry. Vomiting, excruciating pains in the abdominal region and watery diarrhea begin either during the febrile period or after the temperature has fallen. The course may be fulminant; children may have convulsions, become stuporous and die within a few days.

Generalized Tularemia

Ingestion of infected food or tick bites may lead to the typhoidal form. Initial diagnostic suspicion may be meningitis, brucellosis, rickettsial infection or even poisoning. The symptoms are fever, profound toxemia with drowsiness or typhoidal state, abdominal distress and prostration. The gravest prognostic signs are constant delirium or stupor and convulsions with progressive enlargement of the liver and spleen and suppurative inflammation of the meninges and brain.

LABORATORY DIAGNOSIS

Tularemia should be considered in cases of fever in which the child may have been exposed to an infected animal or bitten by an infective

arthropod. Laboratory examination is necessary for confirmation and in many instances for establishment of the diagnosis.

Intradermal Test

On or after the third day of illness intracutaneous injection of 0.05 ml. of a dilute formalin-killed suspension of about 6000 bacteria provokes a specific tuberculin type of reaction after forty-eight hours. Because hypersensitiveness of the skin is a notable feature of tularemia early and late in the disease, this method deserves more frequent use.

Serum Agglutination Test

The macroscopic agglutination test is the standard procedure. Specific antibodies do not appear before the eighth day; they usually appear during the second week and rise to high titers in the third week. They are consequently of little help at the time when the infection is most difficult to diagnose clinically.

Culture and Animal Inoculation

Culture on dextrose cystine or thioglycolate blood agar, or inoculation of guinea pigs or chick embryos with blood or other infected material or of mice with sputum or with blood taken from the heart at autopsy should be made when agglutinins are absent and the course of the disease suggests tularemia.

TREATMENT

Complete rest in bed is necessary during the initial phase. Strict isolation of the patient is unnecessary. Rubber gloves should be worn by those who dress ulcerated lesions and incise and dress suppurating buboes. Sick room attendants need take no precautionary measures beyond ordinary care and cleanliness. The patient's diet should be liquid, high in carbohydrates, semisolid to light as the fever declines, with resumption of full diet as soon as the appetite returns.

Early specific treatment will shorten the disease and increase the patient's chance for recovery. It is of course indicated whenever general tularemia with thoracic or abdominal localization is suspected. Streptomycin and dihydrostreptomycin are the most effective therapeutic agents. Excellent results in the treatment of children have been reported with a daily dose of 1 gm. for five or six days, given in 125-mg. doses at three hour intervals.[8] The response is usually immediate, and the temperature rapidly returns to normal. Severe infections may be treated

with 200 mg. every three hours for two days and then the dose reduced to 100 mg. The total dose in six days need not exceed 5 gm. Relatively large doses are definitely indicated in severe infections. In less severe infections after moderate doses of either of these drugs for two or three days, treatment may be continued for four days with 1 gm. of oxytetracycline in 125-mg. doses at three hour intervals. Since diagnosis in children is difficult, especially when the history is inadequate, a therapeutic trial with streptomycin and Terramycin for severely ill children who live in an endemic area is justified, particularly when the suspected illness fails to respond to other treatment. Until more is known about the efficacy of oxytetracycline, use of this drug alone cannot be recommended. Aureomycin seems to lack the excellent bactericidal property of streptomycin: seven of eight patients treated with this drug underwent recrudescences necessitating use of streptomycin or dihydrostreptomycin.[4] Neither penicillin nor the sulfonamides significantly modify the natural course of the disease.

PASTEURELLOSES SENSU STRICTU

Children may become infected by *Pasteurella multocida*, most often as the result of cat bites. The clinical aspects of the total of ninety-five cases known are incompletely described, but local paronychia-like lesions, general infections with purulent pleurisy, meningitis, myositis, abscess formation, urinary infections, sinusitis and conjunctivitis have been mentioned. Pasteurellae of the hemorrhagic septicemia group have been isolated from the sputum of patients with bronchiectasis. *Pasteurella multocida* may lie dormant in the respiratory tree for months, and only after trauma or some physiologic disturbance of the infected tissue is an acute infection provoked. Localized infection may become generalized and the ensuing illness severe. The diagnosis is usually made by culture or animal inoculation, and the identity of the organism is confirmed by serologic tests. Aureomycin seems superior to oxytetracycline for treatment.[17]

PSEUDOTUBERCULOSIS

Among the seventeen fatal cases of culturally proved human infections with *Pasteurella pseudotuberculosis*, that described by Albrecht[1] is of interest to the pediatrician: The appendix of a fifteen year old child who had had intimate contact with a cat was removed, and inoculation of a guinea pig with a portion of the appendix yielded *P. pseudotuberculosis*. Masshoff[12] has described the occurrence in adolescents of a benign, abscess-forming mesenterial lymphadenitis which, causing symptoms suggestive of acute appendicitis, often misled to surgical interven-

tion. Knapp[7, 7a] has shown by culture, serologic tests or histologic studies of diseased lymph nodes that such local infections may be caused by the pseudotuberculosis bacillus. The serums of fourteen of his fifteen patients agglutinated several strains at titers of 1:200 to 1:3200. The serums of four persons sent in for the Widal test agglutinated at titers ranging from 1:800 to 1:6400. These persons had been hospitalized shortly before, three with signs suggestive of appendicitis, which soon gave way to symptoms of gastroenteritis, and the fourth because of acute gastroenteritis. The occurrence of benign pseudotuberculosis in adolescents deserves attention of pediatricians and laboratory workers.

Fatal cases of this infection have occurred in Seattle, Spokane, San Francisco and New Orleans. They call attention to the existence of this disease in numerous animals in man's environment.[14,22] One culturally identified septicemia caused by *P. pseudotuberculosis* in Czechoslovakia promptly subsided under treatment with streptomycin.

REFERENCES

1. Albrecht, H.: Zur Aetiologie der Enteritis follicularis suppurativa. Wien. klin. Wchnschr., 23:991, 1910.
2. Burroughs, A. L., Holdenried, R., Longanecker, D. S., and Meyer, K. F.: A Field Study of Latent Tularemia in Rodents, with a List of All Known Naturally Infected Vertebrates. J. Infect. Dis., 76:115, 1945.
3. Chen, T. H., and Meyer, K. F.: Studies on Immunization against Plague. VII. A Hemagglutination Test with the Protein Fraction of *Pasteurella Pestis:* A Serologic Comparison of Virulent and Avirulent Strains, with Observations on the Structure of the Bacterial Cells and Its Relationship to Infection and Immunity. J. Immunol., 72:282, 1954.
4. Corwin, W. C., and Stubbs, S. P.: Further Studies on Tularemia in the Ozarks. Review of Forty-Four Cases during a Three-Year Period. J.A.M.A., 149:343, 1952.
5. Francis, E., and Callender, G. R.: Tularemia. The Microscopic Changes of the Lesions in Man. Arch. Path., 3:577, 1927.
6. Girard, G.: Le traitement de la peste pulmonaire par les antibiotiques fungiques. A propos de 25 cas traités à Madagascar. Rev. colon. méd. et chir., 24:174, 1952.
7. Knapp, W., Mesenterial Lymphadenitis Caused by *Pasteurella pseudotuberculosis*. J.A.M.A., to be published.

7a. Knapp, W., and Masshoff, W.: Zur Aetiologie der abszedierenden retikulozytären Lymphadenitis einer praktisch wichtigen, vielfach unter dem Bilde einer akuten Appendizitis verlaufenden Erkrankung. Deutsche med. Wchnschr., 79:1266, 1954.

8. Levy, H. B., Webb, C. H., and Wilkinson, J. D.: Streptomycin Therapy for Childhood Tularemia. New Orleans M. & S. J., 103:78, 1950.
9. Levy, H. B., Webb, C. H., and Wilkinson, J. D.: Tularemia as a Pediatric Problem. Pediatrics, 6:113, 1950.
10. Lillie, R. D., and Francis, E.: I. The Pathology of Tularemia in Man (*Homo Sapiens*). National Institute of Health, Bull. No. 167, 1936, pp. 1–81.
11. Link, V. B.: Plague. Am. J. Trop. Med., 31:453, 1951.
12. Masshoff, W.: Eine neuartige Form der mesenterialen Lymphadenitis. Deutsche med. Wchnschr., 78:532, 1953.

13. McCrumb, F. R., Jr., Larson, A., and Meyer, K. F.: The Chemotherapy of Experimental Plague in the Primate Host. J. Infect. Dis., 92:273, 1953.
14. Meyer, K. F.: Pasteurella, in Dubos, R. J.: Bacterial and Mycotic Infections of Man. 2d ed., Philadelphia, J. B. Lippincott Company, 1952, Chap. 19, pp. 450–487.
15. Meyer, K. F., Connor, C. L., Smyth, F. S., and Eddie, B.: Chronic Relapsing Latent Meningeal Plague. Arch. Int. Med., 59:967, 1937.
16. Mohr, C.: The Plague Problem in the United States. CDC Bull., 9:8, 1950.
17. Neter, E., DeKleine, E. H., and Egan, R. W.: Treatment with Aureomycin of Localized *Pasteurella Multocida* Infection. J. Pediat., 38:242, 1951.
18. Parker, R. R., Steinhaus, E. A., Kohls, G. M., and Jellison, W. L.: Contamination of Natural Waters and Mud with *Pasteurella Tularensis* and Tularemia in Beavers and Muskrats in the Northwestern United States. National Institutes of Health, Bull. No. 193, 1951.
19. Pollitzer, R.: Plague. Geneva, Switzerland: World Health Organization, 1954. (World Health Organization Monograph Series No. 22.)
20. Ramachandran, K.: Treatment of Plague with Aureomycin. J. Indian M. A., 21:217, 1952.
21. Simons, S. A., Stevens, I. M., and Reeves, W. C.: Some Epidemiological Observations on Tularemia in California 1927–1951. Am. J. Trop. Med. & Hyg., 2:483, 1953.
22. Thal, E.: Studies on *Pasteurella pseudotuberculosis*. In manuscript.

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SMALLPOX AND VACCINIA

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Smallpox remains one of the scourges of mankind. Over 400,000 cases occur each year, chiefly in the endemic areas throughout southeast Asia, central Africa and Central America. Widespread travel, especially by air, threatens people with infection in all parts of the world. In its major form, mortality rates are never lower than 30 per cent and, especially in the pediatric age group, are frequently as high as 60 per cent. There is no specific therapy, and none of the antibiotics now available for clinical use has any effect on the vaccinia-variola group of viruses.

ETIOLOGIC AGENTS

Smallpox and vaccinia are caused by two closely related but distinct viruses. The former affects man primarily and is maintained by transmission from man to man. The agent of cowpox is indigenous to cattle and causes a mild disease in cattle or man when infection occurs naturally. Jennerian prophylaxis consists in a deliberate attempt to create an infection (vaccinia) with a living agent (the cowpox virus). On rare occasions this procedure results in serious complications which may lead to death.

SMALLPOX

SOURCE OF INFECTION

Smallpox is transmitted from man to man by contact with a patient or his contaminated surroundings. Virus is present in great quantity in

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the lesions of the skin and mucous membrane, as well as in the dust of the patient's room.

MODE OF TRANSMISSION

Smallpox is as highly transmissible as are measles and chickenpox, and contact need not at all be intimate. Though direct contact predominates as a mode of transmission, indirect contact through articles contaminated by discharge from patients—such as soiled bed clothes, urine, feces or contaminated house dust—has also been a source of infection. There is no known animal reservoir or vector.

PERIOD OF COMMUNICABILITY

The respiratory route undoubtedly predominates as a source of infection. The patient first becomes contagious on the second day of fever, when his respiratory secretions contain virus originating from the mucosa of the nose and mouth. The disease can be transmitted at any time from the first symptoms until all scabs and crusts have been shed, usually thirty days or more after onset of the disease.

INCUBATION PERIOD

This is usually twelve days, with a range from eight to sixteen days.

SUSCEPTIBILITY AND IMMUNITY

Susceptibility is universal, and there is no natural immunity. Age, sex, race and social status play no part in susceptibility. Incidence depends on the number of susceptible persons in the population, the degree of virulence of the prevailing virus strain, and the quantity of exposure. The single most important criterion of susceptibility is the patient's own active immunity provided by recent successful vaccination or a previous attack of the disease.

PATHOGENESIS

During the twelve day incubation period the patient is not infectious. It is likely that primary multiplication of smallpox virus occurs in lymphoid tissues of the respiratory tract, rarely in the incubation period. Next, a transient viremia occurs which results in infection of all cells of the reticuloendothelial system and further multiplication of the virus within these cells. Onset of the febrile illness occurs with liberation of virus for a secondary and more severe viremia, twelve days after infection.[6, 7]

Though the primary viremia has not been demonstrated in man in the early incubation period, virus has been isolated from the blood during the early febrile stages of smallpox. In patients who later die, virus has been found in the blood as long as eight days after the onset of the disease.[8, 17] Virus has also been obtained from throat washings of patients in the pre-eruptive phase of the disease, indicating that the mucosa has become heavily infected. Maculopapular lesions result from the secondary viremia, and all subsequent skin and mucous membrane lesions are a ready source of virus.

CLINICAL PICTURE

The course of a typical case of smallpox in a child is shown in Figure 1.

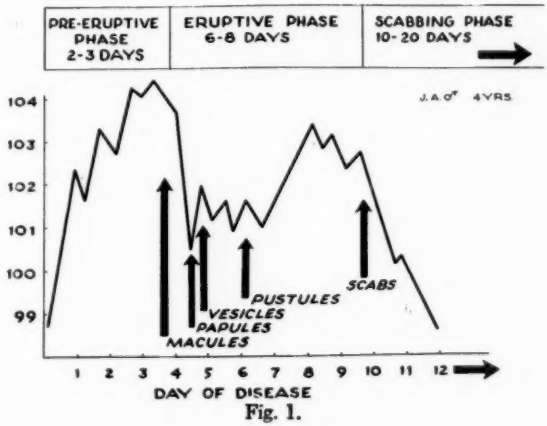

Fig. 1.

The disease begins abruptly with a high fever, headache, malaise and severe generalized muscle aches. During this pre-eruptive phase of the disease a mild erythematous blush may be seen, but this is not common in children. Children appear toxic, refuse food, and may experience febrile convulsions.

The eruptive phase of the disease begins on the third or fourth day of illness, when maculopapular lesions are seen. Characteristically, the patient feels much better, and the temperature drops from 103° or 104° F. to 100° or 101° F. On the fifth or sixth day of illness, papules change into vesicles, which quickly become pustules on the seventh or eighth day (Fig. 2). As pustules form, the temperature usually rises again, although not as high as originally, until drying occurs after the tenth day and the formation of scabs begins (Figs. 3 and 4). In the

eruptive stage a moderate leukopenia is seen, while in the pustular stage leukocytosis occurs.

A secondary rise in temperature in the pustular phase may signal the onset of bacterial complications, including sepsis, pneumonitis or bone

Fig. 2. Fig. 3.

Fig. 4.

Fig. 2. Pustular phase of smallpox.
Fig. 3. Early scabbing phase of smallpox.
Fig. 4. Late scabbing phase of smallpox.

or joint infection. In patients who have been vaccinated or in instances of variola minor a secondary rise in fever may be entirely absent or very mild. The rise is also minimized if penicillin or one of the broad-spectrum antibiotics is used before the sixth day of the disease.

DISTRIBUTION OF LESIONS

In general, the face and extremities and the mucous membranes are most heavily involved, while the trunk and abdomen have fewer lesions.

At any one stage of the disease, most lesions are at the same stage of development.

HEMORRHAGIC SMALLPOX

This is a purpuric type of smallpox which is almost universally fatal and is frequently mistaken for meningococcemia, leukemia or other hemorrhagic diseases. Cases of hemorrhagic smallpox acquire particular importance because the failure to make a correct diagnosis frequently results in a pronounced spread of the disease to the patient's contacts.

Clinical Picture

On the third or fourth day of fever a generalized hyperemic eruption appears and ecchymoses occur in the mucous membranes, the mouth and eyes. Hematuria is frequent, and signs of profound toxemia and hemorrhage leading to death often occur on the third to fifth day of the disease. There is a pronounced neutropenia and thrombocytopenia which may suggest the primary diagnosis of a blood dyscrasia.

The late type of hemorrhagic smallpox is characterized by a maculopapular eruption occurring on the fourth febrile day, which, however, never matures to the vesicular or pustular stage during the subsequent five to nine days. The patient dies on the tenth to fourteenth day of illness, after continued high fever, delirium, hematuria and signs of profound toxemia. The mortality rate of both types of hemorrhagic smallpox is over 95 per cent.

VARIOLA MINOR

Variola minor is a mild disease which may be readily distinguished from the major form on clinical and epidemiologic grounds. The prodromal symptoms of variola minor are much less severe than in the major form of the disease and resemble those of mild influenza. The rash is invariably discrete, and there are fewer lesions than in unmodified variola major.

Variola minor *remains* mild when transferred to contacts, whether they are vaccinated or unvaccinated. Variola major, on the other hand, produces virulent smallpox in unvaccinated contacts, even though their source may be a mild or modified case which is clinically indistinguishable from variola minor, but occurs in the mild form because the patient has been vaccinated successfully.[18] The mortality rate of variola minor is less than 1 per cent.

DIFFERENTIAL DIAGNOSIS

1. In the pre-eruptive phase influenza, malaria, typhoid fever or dengue is frequently suspected.

2. Measles, syphilis or drug rashes are usually considered in the early eruptive phase.

3. Severe chickenpox remains the greatest problem in differential diagnosis of variola major and minor. Characteristically, the distribution of variola is centrifugal and involves the palms and soles. Chickenpox lesions, on the other hand, are usually most heavy on the trunk and abdomen and rarely involve the palms and soles. Chickenpox lesions continue to come out in crops, while variola lesions are roughly at the same stage of development at any given time. Patients suffering from variola characteristically improve, and their fever drops as the eruption appears, but patients suffering from severe chickenpox continue to feel quite sick during the eruptive stage.

4. Hemorrhagic smallpox must be differentiated from meningococcemia, leukemia and other blood dyscrasias, as well as the hemorrhagic fever now prevalent in the Far East.

LABORATORY DIAGNOSIS

A specific diagnosis of smallpox can usually be made in any Public Health laboratory. There are three main diagnostic tests available, each of which has special value, depending on the stage of the disease and the type of specimen that can be obtained from the patient.[16]

1. Smears on clean glass slides from the scrapings of the *base* of smallpox lesions can be examined microscopically for virus elementary bodies. A positive answer may be possible one hour after receipt of the specimen in the laboratory.

2. Complement fixation tests can be used in two ways: (*a*) to detect the presence of antigen in the patient's blood in the first four days of disease or in the skin lesions from the earliest appearance until the last scab falls off; (*b*) to detect antibody in the patient's serum. Results are available within twenty-four hours.

3. Virus isolation is relatively easy on the chorio-allantoic membrane of the embryonated egg, utilizing the patient's blood in the first two or three days of illness (pre-eruptive phase) or material from skin lesions (eruptive phase or scabbing phase). This is the most conclusive test, but results are not obtainable for seventy-two hours.

Collection of Specimens

Blood. Collect 5 ml. of venous blood. Allow it to clot, and separate the serum. Freeze both serum and clots separately for shipment to the laboratory.

Smears. Scrape the base of several lesions with a Bard-Parker blade onto a clean glass slide and allow to air-dry. Smears are of particular value in the papulovesicular phase.

Vesicle or Pustular Fluid. Aspirate several vesicles or pustules, utilizing a tuberculin syringe and a small needle. After obtaining approximately 0.2 cc. of fluid insert

the syringe and needle together with the contents in a large glass tube which is then securely sealed, wrapped and forwarded to the laboratory, preferably in dry ice.

CLINICAL MANAGEMENT

There is no specific therapy for smallpox at present. Penicillin and the broad-spectrum antibiotics are, however, highly effective in preventing the secondary bacterial complications which frequently occur during and after the pustular phase of the disease. Procaine penicillin, 400,000 units per day, intramuscularly, or any of the tetracycline preparations in doses ranging from 25 to 50 mg. per kilogram per day are indicated.

No controlled observations are available on the use of convalescent serum or vaccinia immune gamma globulin. But it is unlikely that the course of the disease would be materially altered by antibody administration once the eruptive stage has occurred. Vaccinia immune gamma globulin may have a place in the prevention or modification of smallpox in an exposed child if it is given in the incubation period or in the pre-eruptive phase of the disease.[14]

In most serious cases of smallpox severe dehydration results because of the pain associated with swallowing. Intravenous fluid therapy may be difficult in the face of massive skin involvement. Increased fluid requirements are associated with any febrile illness, and fluid loss from the skin may be considerable. Oliguria and eventual renal insufficiency may further interfere with the patient's recovery. The use of intensive parenteral fluid therapy or the early institution of feeding through a polyethylene stomach tube holds promise. Boiled skim milk with added carbohydrate (5 to 10 per cent) will supply calories and fluids through the critical ten days of the early eruptive period. With the use of intensive fluid therapy and prophylactic use of antibiotics, mortality rates in the pediatric age group can be reduced to a level between 10 and 30 per cent. All local applications to the skin, or baths, are to be discouraged. It is important, however, to attempt to clean the eyes with warm normal saline solution in order to prevent matting and to decrease the chances of permanent involvement of the sclera.

In severe confluent smallpox and in hemorrhagic smallpox shocklike states have been frequently observed, and this fact leads to the speculation that the future use of intravenous norepinephrine and hydrocortisone may be of value in the management of these patients. Oxygen and, when indicated, digitalis are of value.

COMPLICATIONS AND PROGNOSIS

Bacterial complications include sepsis, pneumonitis, osteomyelitis and septic arthritis. Iritis, corneal ulceration and severe conjunctivitis may all lead to decrease in visual acuity, depending on their severity.

The prognosis of hemorrhagic smallpox is virtually hopeless. The prognosis for survival in children suffering from confluent smallpox is also extremely grave. Children suffering from modified smallpox or discrete smallpox due to previous successful vaccination have a good prognosis, especially if they receive optimal supportive therapy. The over-all mortality rate of the major form of smallpox in children is 30 per cent. In a highly endemic area the death rate from smallpox of vaccinated children between 0 and five years was 23 per cent, while among those not vaccinated, but having no scars, death rates were 55 and 44 per cent, respectively.[19]

CONTROL MEASURES

Isolation. Strict hospital isolation in screened wards is required until all scabs have disappeared.

Concurrent Disinfection. All articles associated with a smallpox patient must be sterilized in high pressure steam or, alternately, by boiling before removing from the patient's room.

Terminal Disinfection. Thorough disinfection of the patient's room is required and would best include painting of the walls and ceilings.

Vaccination. All contacts should be promptly vaccinated or revaccinated and observed for sixteen days from the last day of exposure.

Gamma Globulin. Early evidence indicates that vaccinia immune gamma globulin given to close contacts of patients with smallpox may prevent or modify the disease in the contact.[14] The intramuscular administration of vaccinia immune gamma globulin should follow revaccination by twelve to twenty-four hours with a dose of 0.12 to 0.24 cc. per kilogram of body weight, given once. Smallpox vaccination *after* contact will usually not protect against smallpox unless accompanied by vaccinia immune gamma globulin. Successful vaccination, to insure protection, must be done *before* exposure to a case of smallpox.[12] It is important to ignore history of recent vaccination in the face of exposure unless there is definite evidence of recent scar formation to show that vaccination has, indeed, been successful.

DURATION OF IMMUNITY

For solid protection in an endemic smallpox area, successful vaccination is required each year. Though some immunity persists for five years or longer, repeat vaccination every five to seven years is advisable in nonendemic areas. Where virulent smallpox exists, as many as 80 per cent of victims showed evidence of past successful vaccination, although none did in the previous twelve months.[14]

INVESTIGATION OF SOURCE OF INFECTION

1. The immediate prior case should be sought and all cases of reported chickenpox should be carefully reviewed for errors in diagnosis. *Any* febrile disease during an outbreak may constitute atypical smallpox, and exposed vaccinated contacts may have unrecognized forms of smallpox and thus serve as sources of virulent infection to the unprotected.

2. Once an outbreak has occurred in a community in this country, mass vaccination is generally considered a sound approach. In other areas of the world, stress is placed rather on the supervision and vaccination of all contacts. Enforcement of quarantine of all contacts will prevent further spread, as will the enforced hospital care of all patients and those suspected of the disease, until no longer communicable.

VACCINIA

Jenner's observation in 1798 that naturally acquired cowpox of man protects against smallpox has led to almost universal acceptance of worldwide vaccination programs, with resultant material decrease in the incidence of virulent smallpox.

The widespread vaccination of American children has led to a complete elimination of virulent smallpox in this country, but it has also resulted in a number of serious complications from smallpox vaccination, many of which have had a fatal outcome. In the United States, therefore, medical practitioners dealing with children are much more likely to be involved with the clinical management of cases of vaccinia than of smallpox.

CLINICAL PICTURE

Naturally acquired cowpox of man is a disease of milkers handling infected cattle. Characteristically, one or more vesicles or pustules occur on the hands, and a generalized eruption usually does not appear. Constitutional symptoms and fever are mild, and lesions heal promptly without scarring.[5]

Smallpox vaccination is a deliberate attempt to create an infection with the living virus of cowpox. Immunity does not result from the antigenic material injected, but from the large amount of live virus which develops in the vaccinated person as the result of its propagation in his skin.

Vaccinia in a previously unvaccinated person has a three day incuba-

tion period. It then progresses through the typical stages of papule, vesicle and pustule. An "efflorescence"—an indurated area surrounded by erythema—develops around the lesion between the eighth and fourteenth days. In a previously unvaccinated child, if infection does not take place, there is no reaction other than that from the trauma of vaccination itself.

This initial infection with vaccinia confers not only immunity, but also a bacterial type of skin sensitivity or allergy which is demonstrable as a reaction occurring within forty-eight to seventy-two hours after insertion of inactive or active viral material in anyone having had previous smallpox vaccination or the disease itself.[3] This sensitization, as well as any immunity still remaining, alters the reactions which may develop on repeat vaccinations with potent vaccine. Leake's classification of these, based on measuring the reactions at least three and nine days later, is generally accepted.[15]

The *early reaction,* or *immediate reaction,* in which maximal skin involvement occurs between eight and seventy-two hours after vaccination, is shown by those with immunity so high that there appears to be little or no virus propagation or else rapid recall of immunity, which permits only abortive propagation. Because of this, the term "immune reaction" or "reaction of immunity" is commonly used to designate what may, of course, be merely a sensitization phenomenon which can be obtained with dead as well as live vaccine.[2]

The *vaccinoid* reaction is that seen in persons who retain only partial immunity. Instead of subsiding after seventy-two hours, the immediate reaction which occurs is followed by the appearance of maximal erythematous skin involvement on the fourth to seventh day. Virus propagation always occurs and is terminated by accelerated recall of immunity.

Vaccinia is seen in those who have completely lost effective immunity. The height of the erythematous blush takes place from the eighth to the fourteenth day, and, except for the expected presence of the sensitization reaction within the first three days, the progression is identical with that seen in the first vaccination. It is more difficult to establish infection in previously vaccinated children, since a poor quality vaccine may produce a high percentage of takes after primary vaccination and yet fail to elicit vaccinoid reaction in a group last immunized some years before.

It is safest to regard as a successful vaccination one which results in either vaccinia or a vaccinoid reaction, and to follow an early or immediate reaction by prompt repeat vaccination at another site and perhaps with another lot of vaccine. Successful revaccination can frequently be obtained on the flexor surface of the forearm when the upper arm

appears to be insusceptible. This is especially true if the revaccination site is on or near the previous vaccinations.[12]

SERIOUS COMPLICATIONS OF SMALLPOX VACCINATION

Eczema Vaccinatum

Infants suffering from eczema are peculiarly susceptible to infection with vaccinia virus, and this complication accounts for the largest number of children dying from serious complications of smallpox vaccination. Such children should, therefore, never be vaccinated, even though their lesions appear to be dry, unless they are likely to be exposed to smallpox. They must also be carefully guarded against exposure to

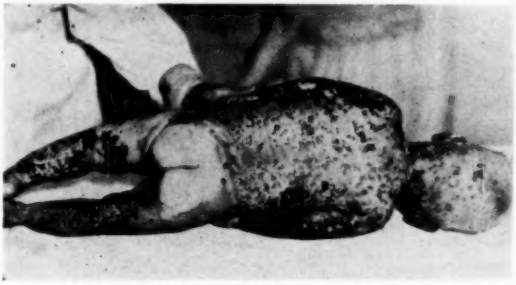

Fig. 5. Eczema vaccinatum. Fatal case.

siblings and other children who have been vaccinated and who may, in turn, infect them.

Lesions first occur in the area of eczema three to four days after exposure. Soon new crops arise in areas of the skin previously uninvolved. Patients are febrile and quite toxic for a period ranging from five to fifteen days. Affected children should be treated as if they were suffering from severe thermal burns. The use of oxygen, blood, plasma and electrolytes, as well as penicillin, is indicated. Recent evidence suggests that there exists a primary defect in the production of specific antibodies against the vaccinia virus. The use of passively administered antibodies in the form of vaccinia immune gamma globulin* may prove to be a valuable adjunct in the specific therapy of this complication.[10] The dose of vaccinia immune gamma globulin is 0.6 to 1.0 cc. per kilogram of body weight.

In infants mortality rates of 40 per cent prevail in this country.

* Vaccinia immune gamma globulin has been prepared and distributed through the financial cooperation of the Blood Program of the American Red Cross and may be obtained by telephoning or wiring Dr. C. Henry Kempe, Department of Pediatrics, University of California Hospital, San Francisco, California (telephone MOntrose 4-3600).

Generalized Vaccinia

Generalized vaccinia occurring in children not suffering from skin disorders is seen when the viremia of primary vaccination gives rise, in turn, to a generalized skin eruption—each lesion simulating a primary vaccination. These frequently continue to come out in crops over a four to six day period, following the onset of the disease, which fre-

Fig. 6. Generalized vaccinia, recovered.

quently coincides with the height of the primary vaccination reaction (eight to fourteen days after vaccination). The mortality rate of generalized vaccinia in the absence of eczema is much lower, since this complication occurs more frequently in children over two years of age. The treatment is identical to that for eczema vaccinatum.

Progressive Vaccinia (Vaccinia Necrosum, Prolonged Vaccinia)

This is an extremely rare complication of vaccination and is characterized by a complete inability to make specific antibodies against the vaccinia virus.[11] This results in the progressive spreading of the primary vaccination take, so that eventually a good part of the arm as well as the rest of the body becomes involved with necrotizing lesions. The systemic reaction is generally mild during the first six weeks of the disease, but progressively increases in the subsequent weeks and eventually leads to death four to six months after onset. This complication is somewhat more apt to occur in cases of congenital agammaglobu-

linemia, but in four recent instances the quantitative gamma globulin levels were normal even though a qualitative defect (dysgammaglobulinemia) existed.

At present there is no way to predict which child is likely to experience this exceedingly rare complication. Since a congenital defect is involved in the failure to make specific antibodies, the logical course of treatment is the provision of large amounts of antivaccinal antibodies

Fig. 7. Progressive vaccinia—successfully treated with vaccinia immune gamma globulin.

in the form of vaccinia immune gamma globulin, given in doses of 0.6 to 1.0 cc. per kilogram of body weight every eight to ten days until lesions are entirely healed.[1] Large amounts of high-titer serum from adults recently successfully vaccinated should be equally effective. Failure to provide large amounts of antibodies will invariably result in death.[4]

Postvaccination Encephalitis

This is a rare complication of smallpox vaccination with an incidence of less than 1 per 200,000 vaccinations.[9] It is virtually unknown in children receiving primary vaccination under the age of three years, and chiefly occurs in school age children whose primary vaccination is done between six and twelve years of age or older. Encephalitic symptoms generally develop at the height of the primary vaccination reaction and not later than the fifteenth day after vaccination. The mortality rate is about 25 per cent, but there is usually eventual physical recovery of those who survive. Behavior disorders are frequent. There is no specific treatment, but this rare complication can best be prevented by vaccination in infancy.[9]

REFERENCES

1. Barbero, J., Gray, A., Scott, T. F. McN., and Kempe, C. H.: Vaccinia Necrosum Successfully Treated with Vaccinia Immune Gamma Globulin. To be published.
2. Benenson, A. S., and Kempe, C. H.: Immunologic Response to Vaccination with Inactivated Cowpox Vaccine. Federation Proc., *10*:402, 1951.
3. Benenson, A. S., Kempe, C. H., and Wheeler, R. E.: Problems in Maintaining Immunity to Smallpox. Am. J. Pub. Health, *42*:535, 1952.
4. Bigler, J. A., and Slotkowski, E. L.: Smallpox Vaccination with Prolonged Vaccinia. Pediatrics, *7*:24, 1951.
5. Davis, J. H. T., James, L. R., and Downie, A. W.: Infection in Farm Workers. Lancet, *2*:1534, 1938.
6. Downie, A. W.: Jenner's Cowpox Inoculation (Jenner Memorial Lecture). Brit. M. J., *2*:251, 1951.
7. Downie, A. W.: The Dynamics of Virus and Rickettsial Infections. International Symposium. New York, Blakiston Company, Inc., 1954, p. 194.
8. Downie, A. W., McCarthy, K., and MacDonald, A.: Viraemia in Smallpox. Lancet, *2*:513, 1950.
9. Greenberg, M., and Appelbaum, E. A.: Postvaccinal Encephalitis. Am. J. M. Sc., *216*:565, 1948.
10. Kempe, C. H.: Serious Complications of Smallpox Vaccination: Early Clinical Use of Vaccinia Immune Gamma Globulin. To be published.
11. Kempe, C. H.: Progressive Vaccinia: Report of Six Recent Cases. To be published.
12. Kempe, C. H.: Newer Immunologic Concepts of Smallpox Vaccination. To be published.
13. Kempe, C. H., and Benenson, A. E.: Vaccinia. Passive Immunity in Newborn Infants. J. Pediat., *42*:525, 1953.
14. Kempe, C. H., and Berge, T. O.: Vaccinia Immune Gamma Globulin in the Prevention and Treatment of Smallpox. To be published.
15. Leake, J. P.: Questions and Answers on Smallpox and Vaccination. Reprint 1137 from Public Health Reports (revised 1946).
16. MacCallum, F. O.: The Dynamics of Virus and Rickettsial Infections. International Symposium. New York, Blakiston Company, Inc., 1954, p. 325.
17. MacCallum, F. O., McPherson, C. A., and Johnstone, D. F.: Laboratory Investigation of Smallpox Patients, with Particular Reference to Infectivity in Early Stages. Lancet, *2*:514, 1950.
18. Marsden, J. P.: A Personal Analysis of 13,686 Cases. Bull. Hyg. London, *23*: 735, 1948.
19. Pandit, C. G., and Masilamani, S. E.: Personal communication to C. H. Kempe.

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CAT SCRATCH DISEASE

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Cat scratch disease was first recognized in the early 1930s by Lee Foshay[5] in the United States and by Robert Debré[8] in France as an infectious disease with regional lymphadenitis. In 1945 Hanger and Rose[5] made a skin test antigen from pus from a human cat scratch disease lymph node which gave positive intradermal reactions with other cases. Similar antigen was forwarded to Debré in France, who found that cat scratch disease patients there had positive skin tests. Debré[10] published the first paper on cat scratch disease in 1950. Roughly over 400 cases have been reported in the world literature by this time. The most extensive summary and bibliography is that of Debré and Job.[9]

EPIDEMIOLOGY AND ETIOLOGY

The disease is worldwide. There is no sex or seasonal predilection, and it occurs in white, yellow and black races. All ages are involved, the increased frequency in children probably being due to their more intimate contact with cats.

The infectious agent has not been successfully defined. However, there seems to be little question that this is an infectious disease, or a group of such diseases. Mollaret[25] was able to produce granulomatous lesions in skin and lymph nodes of three monkeys with ground-up lymph node from two cases of cat scratch disease. The monkeys became skin test positive. He was unable, however, to demonstrate adequately the transmission of the monkey lesions to a second and third monkey. On the fourth of a series of inoculations of human cat scratch disease lymph node pus into a laboratory technician, Mollaret[25] produced the clinical picture of the disease. No one else has been able to completely confirm these findings of Mollaret.

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The proposed etiologic agent has stubbornly resisted any other means of culture or animal inoculation. Most workers tentatively accept it as a virus because of three findings: (1) sterile cultures of infected lymph nodes; (2) the presence in human cat scratch disease serum of low complement fixation titer against the psittacosis-lymphogranuloma group;[26] (3) granular corpuscles, thought to be elementary bodies of virus, found in cat scratch disease granulomas in man and monkey.[25] Since these granular corpuscles occur in both viral and nonviral diseases,[39] they have no real significance here.

Nothing is known of the true source of the infection. One thing that stands out is the role played by cats in the disease transmission. Some writers minimize this and use the name "benign inoculation lymphoreticulosis" instead of cat scratch disease. In 300 reported cases[9] 83 per cent showed cat contact divided as follows: 54 per cent with cat scratch, 6 per cent with cat bite and 13 per cent with other inoculation trauma associated with cat contact. The remaining 17 per cent had inoculation trauma of other type, thorn, splinter, chicken scratch, and the like, or no known inoculation, without cat contact. The few family epidemics reported[6, 9] have always had a cat associated with the family. One endemic area is found in a town where the French National Veterinary College is located.[11] Gifford[16] has shown significantly positive skin tests in veterinarians and suggestion of hyperergy in this group.

Throughout their period of transmission potential, which lasts from several days to several weeks,[9] the cats remain healthy. Indeed, if a cat is sick or dies, one should look for another disease. Skin tests of carrier cats are uniformly negative. It has not been possible to isolate the feline pneumonitis virus of Baker from cat scratch disease human lymph node material,* although this has been mentioned as a possible etiologic agent.[4] Because of the predatory habits of cats, rodents and birds have been mentioned as possible reservoirs of infection. Findings are completely lacking, however. The presence of the virus on cat claws, in cat saliva and on cat fur has been postulated, but no isolation has been made directly from the cat.

CLINICAL APPEARANCE

Visible lesions produced are of two types: first, the inoculation site, present in roughly half of the cases; second, the regional lymph node enlargement. The inoculation lesion varies from an inflamed scratch mark, which may show suppuration, to an erythematous papule surmounted by a vesicle, pustule or a little crust. The lesion may be present or absent by the time of lymph node enlargement, or it may

* H. Gifford: unpublished data.

reappear as the lymph nodes are maximally involved. The lymph node swelling follows the inoculation injury by a variable length of time ranging anywhere from a few days up to a few months. The customary time, however, is two to four weeks. The swollen lymph nodes may remain movable and nontender, or they may become painful, warm, and fixed to the surrounding tissue and to one another, with reddening of the overlying skin. Typically the first and sometimes the second group of lymph nodes draining the area of inoculation are involved. Rarely is there adenopathy beyond this, or diffuse adenopathy throughout the body.[21, 36] In lymph nodes most severely involved suppuration may occur, the nodes often becoming fluctuant. The nodes may rupture spontaneously, and draining sinus tracts may develop. These heal spontaneously with time and leave little in the way of scarring.

The basic lesion is a granuloma, which is present in both the inoculation site and regional lymph nodes. Time is necessary for full development of this, and early stages may show only nonspecific inflammatory changes and hyperplasia. The lymph nodes, which are usually soft, vary on cut surface, sometimes being grayish-red and homogeneous, sometimes showing scattered foci of necrosis, and other times being merely a bag of pus. In focal areas in the sinusoids and within germinal centers of lymph node follicles, small clusters of epithelioid cells appear. These are soon infiltrated by polymorphonuclear leukocytes and lymphocytes in few to moderate numbers. Necrosis of both the epithelioid cells and inflammatory cells occurs, producing a small central abscess. Around this central abscess there is palisading of epithelioid cells, and in turn around the epithelioid zone there is a cuff of lymphocytes with variable numbers of plasma cells and polymorphonuclear leukocytes. An occasional multinucleated giant ~~cell of either~~ Langhans type or foreign body type may be seen, usually in the palisaded epithelioid areas. These foci of granulomatous reaction may be round, oval or somewhat stellate. They often coalesce to form large suppurative areas within the lymph nodes. The process may stop at any stage, and regression is the usual course. The histologic changes are not specific for this disease, but are characteristic and suggestive of it. Appropriate stains and cultures should be made to rule out the presence of bacteria and fungi.

The clinical picture is a basically simple one of malaise, fever and local lymphadenitis. Also reported with significant frequency are nausea, anorexia, weakness, aching, chills or chilly sensations, and headache. The fever is variable as to presence, duration and course. It may be septic and as high as 104° F. The patient is often not nearly as ill, however, as the severity of the lymph node involvement would suggest he should be. A few instances of early, short-lived macular or papular rash have been described.

Of importance is a recognition of atypical forms or variations of the disease pattern. The difference lies mostly in localization, since the fundamental change in all instances, with the possible exception of the thoracic and nervous system types, is one of granulomatous lymphadenitis. The most common lymph nodes involved are the cervical group, the epitrochlear and axillary groups, and the femoral and inguinal groups, roughly in that order. Uncommonly the cutaneous lesion may dominate the picture.[19] It is a significant feature in all types that lymphangitis is not present, but the regional lymph nodes are swollen. One may conveniently classify the variations of this disease as follows:

1. Inapparent forms
2. Typical forms
   A. Purely glandular
   B. Purely cutaneous
   C. Mixed
3. Atypical forms
   A. Pseudoveneric
   B. Erythema nodosum
   C. Ocular
   D. Anginal
   E. Mesenteric
   F. Thoracic
   G. Nervous system

The inapparent forms have appeared in a few family epidemics and are those cases in which the skin test turns positive, without clinical signs or symptoms, when the patient is closely associated with a typical case and with the involved cat.[9] The pseudoveneric forms[7, 13, 29] are merely ones of inguinal gland involvement. At least seven reports[3, 7, 14, 17, 27, 31, 37] of erythema nodosum associated with cat scratch disease have appeared. The ocular type[1] typically produces Parinaud's syndrome with conjunctivitis with preauricular lymph node enlargement. At least three cases of anginal type have been reported.[5, 23, 34] Mollaret et al.[23] presented one with pharyngeal abscess and involvement of cervical lymph nodes. Usteri[34] reports cases in which laparotomy after a clinical diagnosis of appendicitis showed no appendiceal changes, but enlargement of mesenteric lymph nodes and positive skin test reactions to cat scratch disease antigen. The microscopic changes in the mesenteric lymph nodes were characteristic. The rare thoracic type shows enlargement of mediastinal lymph nodes.[2, 22] Two cases of a pulmonary type have been reported,[33, 34] showing a lung infiltration like that of atypical pneumonia, associated with enlarged mediastinal lymph nodes.

The anginal, mesenteric and thoracic types usually occur without skin inoculation, so that a mucosal portal of entry must be assumed.

Eleven reported cases of the nervous system type have been reviewed

by Debré.[9] In any one of these cases signs and symptoms predominated to classify it as meningitis, encephalitis, myelitis or neuritis. There were no fatalities and no histologic examinations. It is unwise to diagnose such clinical manifestations as cat scratch disease in the presence of a positive skin test unless there is a recent or active typical inoculation lymph node complex, since the positive skin test may be the result of past contact with the disease.

The protean manifestations of this disease make it likely that other types will be described in the future. Already a case of acute thyroiditis thought to be caused by cat scratch disease has been reported.[28]

SKIN TEST AND LABORATORY PROCEDURES

The skin test is thought to be relatively specific,[4, 5, 35] cases of presumed viral urethritis being the only exception reported so far.[38] The antigen is made from bacteriologically sterile pus from human cat scratch disease lymph nodes by diluting the pus 1:5 with sterile saline solution and heating from 56° to 60° C. for one hour on each of two successive days. Heating much beyond this will diminish or destroy antigenicity.* The strength of different antigens varies, so that no arbitrary measurement may be set on the diameter of erythema or induration obtained when 0.1 ml. of the antigen is injected intradermally. Ground-up human cat scratch disease lymph node may be used to make antigen, but it is thought by some to be less potent. There are apparently different strains of the etiologic agent,[5, 26] and one antigen may not react with all cases of cat scratch disease. The antigen is not available commercially. One may make one's own or obtain it from someone working with the disease. Antigen should be tested on known reactors and on negative controls. Pus to be used for antigen should be withdrawn before skin tests are made (P. Mollaret†). The antigen is stable, best kept in the frozen state, and will not be injured by repeated thawing and refreezing.

The reaction to the skin test is a delayed one. There is often an immediate nonspecific reaction which fades within hours. A true reaction appears in a variable period of time, usually within forty-eight hours. Since reactions may occur as late as the sixth to seventh day after injection, it is wise to check the reaction in forty-eight hours and one week, and to have the patient observe and report any change during the interim. The skin test usually fades within a matter of days; however, occasional ones may undergo ulceration and heal over a period of weeks rather than days. A few positive skin tests last for months (Mollaret and W. B. Daniels†), and these may represent hyperergic reactions. Skin test reactivity may last five years or more.

* H. Gifford: unpublished data.

† Personal communication to the author.

Other laboratory work is variously reported, but is usually not remarkable with the exception of an often increased sedimentation rate[7, 9] and uncommon instances of leukocytosis[9, 18] or leukopenia.[9, 18, 20, 36] Laboratory procedures are usually used to exclude other diseases in the differential diagnosis. Serum may be tested for complement-fixing antibodies against members of the psittacosis-lymphogranuloma group. Mollaret[26] showed that these complement-fixing antibodies appeared late in the disease, and only in those cases with necrosis of the lymph node tissue. The titers obtained for the most part were low. In a few instances patients showing no titer developed a low titer on second serum sample drawn a week or so later. This test has limited practical value, but is helpful when combined with a negative Frei test if specific cat scratch disease skin test antigen is not available.

DIAGNOSIS, COURSE AND TREATMENT

The diagnosis of cat scratch disease should consider the following: cat contact, inoculation trauma and lesion, absence of lymphangitis, regional lymphadenitis, a well-being not compatible with the severity of lymph node involvement, and a positive skin test. Negative cultures and a microscopic examination of lymph nodes are confirmatory. The differential diagnosis should consider all other possible causes of lymphadenopathy.

The disease is benign and self-limited. Its course is variable, and prolonged involvement up to several months has been recorded.

Therapy is not specific, although the use of the following drugs has been suggested as a probable means of shortening the course: Aureomycin,[5, 9] erythromycin,[15] Terramycin,[5, 24, 36] chloramphenicol.[5, 7] The lack of response to these drugs may be used to some extent as a diagnostic point, since it is not uncommon for the disease process to continue in the face of such antibiotic therapy. In such an instance it may be necessary either to aspirate a fluctuant lymph node or surgically remove a lymph node or group of lymph nodes in order to interrupt the disease process. Surgical removal usually is followed by healing.[9, 12, 36]

The *prognosis* is exceedingly good. There are apparently no sequelae.

One case in which death is alleged to have been due to cat scratch disease has been reported.* The evidence offered in substantiation of this is wholly inadequate. However, the possibility of cat scratch disease causing death in future cases cannot be completely dismissed, particularly in the central nervous system types. Each reported case should

* Gräff, S.: Tod an Katzenkratzkrankheit (Felinose). Monatsschr. Kinderh., *102*: 232, 1954.

be subjected to rigid evaluation, since the experience to date indicates that this is a nonfatal disease.

REFERENCES

1. Cassady, V. J., and Culbertson, C. S.: Cat Scratch Disease and Parinaud's Oculoglandular Syndrome. Arch. Ophthal., 50:68, 1953.
2. Charbonneaux, H.: Quatre cas de maladie des griffes du chat à Montreal. Union Méd. Canada, 81:123, 1952.
3. Cox, P. J. M.: Cat Scratch Fever. Proc. Roy. Soc. Med. (Ped.), 45:456, 1952.
4. Daeschner, C. W., Salmon, G. W., and Heys, F. M.: Cat Scratch Fever. J. Pediat., 43:371, 1953.
5. Daniels, W. B., and MacMurray, F. G.: Cat Scratch Disease. Arch. Int. Med., 88:736, 1951.
6. Daniels, W. B., and MacMurray, F. G.: Cat Scratch Disease; Non-bacterial Regional Lymphadenitis: A Report of 60 Cases. Ann. Int. Med., 37:697, 1952.
7. Daniels, W. B., and MacMurray, F. G.: Cat Scratch Disease. J.A.M.A., 154: 1247, 1954.
8. Debré, R.: La maladie des griffes de chat. Marseille méd., 87:375, 1950.
9. Debré, R., and Job, J. C.: La maladie des griffes du chat. Acta paediat., 43: March, 1954, Suppl. 96.
10. Debré, R., Lamy, M., Jammet, M. L., Costil, L., and Mozziconacci, P.: La maladie des griffes de chat. Bull. et mém. Soc. méd. d. hôp. de Paris, 66:76, 1950.
11. Debré, R., Van Bogaert, L., Thieffry, S., and Arthuis, M.: Accidents nerveux de la maladie des griffes du chat. Bull. Acad. Nat. Méd., 136:454, 1952.
12. Denechau, D.: Un nouvel exemple de maladie des griffes de chat. Excision du foyer et du ganglion infectés. Bull. et mém. Soc. méd. d. hôp. de Paris, 68: 347, 1952.
13. Duperrat, B.: Forme pseudovénerienne de la maladie des griffes de chat. Bull. et mém. Soc. méd. d. hôp. de Paris, 67:848, 1951.
14. Fontan, A., Verger, P., Martin, C., and Verin: Sur un cas de lymphoréticulose bénigne d'inoculation. J. Méd. Bordeaux, 128:1027, 1951.
15. Gennes, L. de, Bricaire, H., Tourneur, R., Weill-Fage, J. C., and Aubert, P.: Sur quatre observations d'infections traitées par l'erythromycine. Bull. et mém. Soc. méd. d. hôp. de Paris, 69:377, 1953.
16. Gifford, H.: Skin Test Reactions in Cat Scratch Disease. Proc. Lane Medical Society, Stanford Med. Bull., to be published.
17. Greer, W. E. R., and Keefer, C. F.: Cat Scratch Fever: A Disease Entity. New England J. Med., 244:545, 1951.
18. Gsell, O., Forster, R., and Klaus, E.: Virus-Kratz-Lymphadenitis. Schweiz. med. Wchnschr., 81:699, 1951.
19. Hedinger, C., Usteri, C., and Wertman, F.: Der kutane Primaräffekt der sogenannten Katzenkratzkrankheit, einer benignen virus Lymphadenitis. Dermatologica, 104:101, 1952.
20. Lemaire, A., and Debray, J.: Sur une épidémie familiale d'adénite subaiquë bénigne de nature incommue. Bull. et mém. Soc. méd. d. hôp. de Paris, 66: 375, 1950.
21. Mahoudean, D., Lemperiére, and Leroux, G.: Un nouveau cas de polyadénopathie subaiquë paraissant consécutive à de griffures de chat. Bull. et mém. Soc. méd. d. hôp. de Paris, 66:410, 1950.
22. Meyer, A., and Fréour: Adenopathies médiastinales transitoires coexistantes avec une reaction positive à l'antigene de la lymphoréticulose bénigne d'inoculation. Soc. Fr. Path. Resp., January 10, 1954. Cited by Debré.[4]
23. Mollaret, P., Maduro, R., and Chevance, L. G.: La forme pharyngée de la lympho-

réticulose bénigne d'inoculation. Bull. et mém. Soc. méd. d. hôp. de Paris, 67: 565, 1951.
24. Mollaret, P., Reilly, J., Bastin, R., and Tournier, P.: Sur une adénopathie régionale subaiguë et spontanément curable, avec intradermo-reaction et lesions ganglionnaires particuliéres. Bull. et mém. Soc. méd. d. hôp. de Paris, 66:424, 1950.
25. Mollaret, P., Reilly, J., Bastin, R., and Tournier, P.: Sur découverte du virus de la lymphoréticulose bénigne d'inoculation. Press. méd. 59:701, 1951.
26. Mollaret, P., Reilly, J., Bastin, R., and Tournier, P.: Lymphoréticulose bénigne d'inoculation et caractérisation du virus par sérologie et colorations. Bull. et mém. Soc. méd. d. hôp. de Paris, 67:693, 1951.
27. Roux, J. A.: Un cas d'erytheme noueux dans la maladie des griffes du chat. Pédiatrie, 41:238, 1952.
28. Shumway, M., and Davis, P. L.: Cat Scratch Thyroiditis Treated with TSH. J. Clin. Endocrin. & Metab., 14:742, 1954.
29. Siquier, F., Welti, J. J., and Lumbroso, P.: Nouvelles observations de formes pseudo-vénériennes de la maladie des griffes du chat. Bull. et mém. Soc. méd. d. hôp. de Paris, 68:216, 1952.
30. Stevens, H.: Cat Scratch Fever Encephalitis. Am. J. Dis. Child., 84:218, 1952.
31. Thiers, H., and Thivolet: Erytheme noueux au cours de l'évolution d'une lymphoréticulose bénigne d'inoculation. Lyon méd., 187:10, 1952.
32. Thompson, E., and Miller, K. F.: Cat Scratch Encephalitis. Ann. Int. Med., 39: 146, 1953.
33. Turiaf, J., and Jeanjean, Y.: Le syndrome pneumoganglionnaire de la lymphoréticulose bénigne d'inoculation. Bull. et mém. Soc. méd. d. hôp. de Paris, 69: 914, 1953.
34. Usteri, C., Wegmann, T., and Kedinger, C.: Ueber atypische Formen der sogenannten Katzenkratzkrankheit, einer benignen Virus-lymphadenitis. Schweiz. med. Wchnschr., 82:1287, 1952.
35. Waters, W. J., Kaher, S. S., and Prior, J. T.: Cat Scratch Syndrome. Pediatrics, 10:311, 1952.
36. Wegmann, T., Usteri, C., and Hedinger, C.: Die sogenannte Katzenkratzkrankheit, eine benigne Viruslymphadenitis. Schweiz. med. Wchnschr., 81:853, 1951.
37. Weill, J., and Blas, F.: Erytheme noueux et griffure du chat. Bull. et mém. Soc. méd. d. hôp. de Paris, 67:1141, 1951.
38. Wilcox, R. R.: Letters to Editor. Lancet, 2:470, 1952.
39. Winship, T.: Pathological Changes in So-Called Cat-Scratch Fever. Am. J. Clin. Path., 23:1012, 1953.

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RAMSAY HUNT SYNDROME

(GENICULATE ZOSTER)

A Rare Complication of Herpes of the Central Nervous System, with a Note on Herpes Simplex

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Among the rare neurologic diseases of childhood are the complications of virus herpes, simplex or zoster. Usually these are benign and present themselves as simple skin lesions.

The central nervous system complication of herpes simplex, though rare, can cause a generalized encephalitis which may be fatal. The type of encephalitis produced shows itself histologically to be the inclusion type, and the neurons contain the usual acidophilic intranuclear inclusions (type A). The leptomeninges as well as the perivascular spaces show mononuclear infiltrations. Other evidence of inflammatory processes may also be seen. Virus may be isolated from the brain by the usual techniques.[3]

As is herpes simplex, herpes zoster is seldom fatal, and usually presents a disturbance in the sensory elements of the central nervous system. Thus herpes zoster usually involves an isolated spinal or cranial sensory ganglion, the posterior gray matter of the spinal cord and/or the adjacent leptomeninges.[2] The virus may spread from the posterior to the anterior horns in these cases and cause muscular paralyses which appear from time to time. Herpes zoster may occur as an isolated illness or as a complication of many other debilitating diseases.[1] Among others, the so-called geniculate herpes, a protean manifestation of herpes zoster, is known as the Ramsay Hunt syndrome.

Between 1906 and 1910 Hunt reviewed fifty-six cases, four of which were his own. He pointed out the relationship between the herpetic lesion and the facial nerve. The motor and sensory portions of the facial

nerve were involved in the presence of the lesion. He noted also involvement of the eighth cranial nerve and gasserian ganglion as part of this syndrome. The syndrome is usually associated with a skin lesion of the external ear, and may present, either partially or totally, the following clinical picture:

CLINICAL MANIFESTATIONS

Subjectively, the patient may complain that sounds cause pain and produce hyperacousis (phonophobia). He may complain also of tinnitus or vertigo, or both.

On examination one usually finds the skin lesions or evidence of their having been present. The usual site of these lesions involves the external ear within the antitragus, the incisura intertragica, and extends onto the surface of the tympanic membrane. The syndrome may appear without involvement of the geniculate zone of the ear, but associated with other herpetic lesions; that is, occipitocervical herpes, trigeminal herpes, or herpes involving other dermatomes of the body.[2, 6, 8] These lesions are frequently accompanied by severe pain and fever.

Among the other objective findings there is total facial paralysis of the motor component involving all branches. Usually there is total loss of taste for the anterior two thirds of the same side of the tongue. There may be overtearing, which produces "crocodile tears," the condition being due to paralysis of the lacrimal duct muscles. However, a dry eye may be present. There may be loss of hearing for the ipsilateral side as well as labyrinthine disturbances manifested by vertigo, nystagmus and other associated signs. There may be disturbance of swallowing due to the paralysis of the palate. Signs of leptomeningeal irritation such as headache, stiff neck, and occasionally increased intraspinal pressure may be present.[1, 4]

LABORATORY STUDIES

Diagnostic laboratory studies should include specific serologic tests for virus. In view of the meningeal response, a lumbar puncture should be done. The cerebrospinal fluid is usually characterized by an increase of total proteins and white cells, and constitutes a picture of mixed lymphocytes and polymorphonuclear leukocytes, the former predominating.[5] Spinal fluid sugars are usually normal, but chlorides may be reduced. The following case illustrates the salient features of this syndrome:

CASE REPORT. R. O., a white boy aged 13, was first seen on October 24, 1952, because of a right facial paralysis of some 8 days' duration. The patient's mother reported that the boy had been well until the first part of October, when he had a

"runny nose," pain in his right ear, and was thought to have a "cold." There was some inflammation of his right eye. One other member of the family was ill at the same time.

All this cleared, but on October 17, 1952, the patient showed facial asymmetry, with progressive weakness for all muscles of the right half of the face. External examination of the ear failed to reveal any lesions. The right eye watered excessively (crocodile tears). There were no other disturbances, however, such as those of swallowing, hearing or vision.

Neurologic examination revealed the following: flattening and loss of expression of the right half of the face; inability to contract the various facial muscles. The right pupil was larger than the left; Bell's eye phenomenon was present. There was loss of taste to salt, sugar and vinegar for the anterior two thirds of the right half of the tongue. There was no olfactory disturbance. The rest of the cranial nerves were intact, without evidence of hyperacousis (phonophobia).

Examination of the reflexes revealed an increased response in the left triceps, biceps, quadriceps and gastrocnemius muscles. There were no other pyramidal, extrapyramidal, cerebellar, labyrinthine or sensory signs.

In view of the clinical picture, a diagnosis of Ramsay Hunt syndrome, postinfectious, probably herpetic in origin, was made.

Unfortunately, extensive diagnostic studies, i.e., lumbar puncture and other laboratory procedures, were not done because family permission could not be obtained.

Progressive recovery in both sensory and motor portions of the facial nerve characterized the clinical course, so that by February 4, 1953, all evidence of facial paralysis and sensory disturbance in taste had disappeared.

DISCUSSION

The frequency of facial paralysis associated with a herpetic lesion is probably greater than recorded in the literature, since frequently these cases are seen, but not reported. Of those cases reported, however, occurrence of the Ramsay Hunt syndrome is rare in children. Although Hunt reported fifty-six cases in patients between 1906 and 1910, none of the patients were children. Subsequently only O'Neill[7] reported that two of his eight patients were children, and stated also that Kolb at the Mayo Clinic saw but one child who suffered with this illness. In our own series of twelve cases of facial paralysis, the only child seen is the one reported here. O'Neill reports the youngest child in whom the illness had its onset at six months.

Herpes zoster occurs more often in childhood than is generally realized, and with greater frequency in the ophthalmic branch of the trigeminal nerve, manifesting itself in complications of total external and internal muscular ocular paraylsis, glaucoma, iritis and keratitis.[1]

DIFFERENTIAL DIAGNOSIS

Differential diagnosis in the Ramsay Hunt syndrome should be based first on the exclusion of ordinary eczema, pityriasis rosea and related conditions. Its paralytic manifestation calls for the consideration of birth injury, cerebrovascular accidents of childhood, simple Bell's palsy, congenital disturbances, absence of a nerve, and especially branchial

cleft problems, which may mimic the syndrome. Besides these, cerebellar pontine angle growths which may involve the trigeminus with consequent disturbance of hearing and labyrinthine function, should be excluded. Poliomyelitis, the Guillain-Barré-Strohl syndrome and infectious mononucleosis all may produce a condition similar to that seen in the Ramsay Hunt syndrome. In our cases of facial paralysis two adults had a similar syndrome associated with a diabetic neuropathy.

TREATMENT

The methods of treatment in this condition are multitudinous and varied. Such odd medications as poultices, salves, heat and witchcraft all are said to have been effective! Large doses of vitamins, especially of B complex, with heavy emphasis on B_{12} in daily doses of 1000 micrograms given over a ten to fifteen day period, have been tried with equivocal results. Protamide falls into the same classification, and the same indefinite results have been obtained with its use.

One must remember that this illness is a self-limiting disease. The therapy which has proved most valuable is Aureomycin, as an antibiotic, and x-ray directed to the lesions and ganglia involved. This latter should be given both in the early and late stages in 100 to 400 roentgen depth dosage. The use of galvanic stimulation applied to the facial muscles seems to be beneficial. Face splints can be utilized; frequent massage of the face by the patient to maintain muscle "tone" is suggested.

To date, however, there has been no definite antibiotic or specific drug for the relief of pain or paralysis or for the prevention of complications that occur in geniculate zoster (Ramsay Hunt syndrome).

PROGNOSIS AND COMPLICATIONS

Prognosis and complications interweave in the final outcome. Basically, the disease will terminate in one of three ways: (1) complete recovery without complications; (2) recovery in which there may be partial to complete return of function for all but the affected aspect; (3) total disability in every phase of the syndrome.[2, 7]

The complications that may arise include a total and permanent loss of taste for the ipsilateral anterior two thirds of the tongue; total paralysis of the face; deafness; chronic labyrinthitis which may produce bouts of vertigo simulating Ménière's syndrome. A rare complication is that of hemifacial spasm, often referred to erroneously as "facial tic." The spasm is both painful and disabling, and at times patients will resort to surgical intervention.[9]

SUMMARY AND CONCLUSIONS

1. Herpes simplex and herpes zoster can create neurologic disturbances.

2. Herpes simplex may produce a fatal encephalitis of the inclusion type, while herpes zoster can produce what is commonly known as a posterior poliomyelitis, and in rare instances the Ramsay Hunt syndrome of total facial paralysis, sensory loss of taste in the anterior two thirds of the tongue, loss of hearing, and disturbance of equilibrium.

REFERENCES

1. Baird, P. C., Jr.: Herpes Zoster. New England J. Med., 228:568, 1943.
2. Denny-Brown, D., Adams, R. D., and Fitzpatrick, P. J.: Pathologic Features of Herpes Zoster (a Note on "Geniculate Herpes"). Arch. Neurol. & Psychiat., 51:216, 1944.
3. Ginder, D. R., and Whorton, C. M.: Herpes Simplex Encephalitis. J. Pediat., 39: 298, 1951.
4. Hunt, J. R.: On Herpetic Inflammation of the Geniculate Ganglion. A New Syndrome and Its Complication. J. Nerv. & Ment. Dis., 34:73, 1907.
5. Johnson, L. F., and Zonderman, B.: Herpes Zoster Oticus (Ramsay Hunt Syndrome). Arch. Otolaryngol., 48:1, 1948.
6. Leff, W. A.: Ramsay Hunt Syndrome with Thoracic Herpex. J. M. Soc. New Jersey, 44:262, 1947.
7. O'Neill, H.: Herpes Zoster Auris ("Geniculate" Ganglionitis). Report of 8 Cases with Photographs of the So-Called Syndrome of Ramsay Hunt. Arch. Otolaryngol., 42:309, 1945.
8. Tschiassny, K.: The Site of the Facial Nerve Lesion in Cases of Ramsay Hunt's Syndrome. Ann. Otol., Rhin. & Laryngol., 55:152, 1946.
9. Wartenberg, R.: Hemifacial Spasm. New York, Oxford University Press, 1952, p. 10 ff.

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THE ACUTE ASEPTIC MENINGITIS SYNDROME

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Unlike the other clinics of this issue, this one portrays a symptom complex of multiple etiology rather than the manifestations of a single infectious agent. Accurate figures of its incidence among children are not available; personal experience suggests that it is not really unusual.

CLINICAL PICTURE

To be certain that we understand each other, let me state what I mean by the acute aseptic meningitis syndrome: In the course of one or two days, fever, nausea or vomiting in a previously healthy child develops; he may be irritable, but is not fidgety, preferring to lie quietly and complaining of neck and back soreness on moving about. When he is examined, there is nuchal and spinal rigidity, but no abnormal or absent reflexes, nor can any localizing neurologic signs be found; no pathognomonic physical signs can be elicited such as the parotitis of mumps, the paresis of poliomyelitis, the jaundice which may accompany leptospirosis or infectious mononucleosis. The white blood cell count is essentially normal. A lumbar puncture is done, revealing from 30 to 3000 cells per cubic millimeter, predominantly lymphocytic, free of organisms on direct smear and with a sugar content relatively normal or slightly elevated. This clinical picture is the acute aseptic meningitis syndrome and will make up the body of this clinic (Table 1). If a specific diagnosis and prognosis are desired, it is extremely important to collect 8 to 10 ml. of *sterile clotted blood immediately* and have the serum separated and kept refrigerated for special tests to be mentioned later.

In any given instance the etiology may become clear as the clinical

TABLE 1. *Acute Aseptic Meningitis Syndrome*

CLINICAL CHARACTERISTICS	CEREBROSPINAL FLUID CHARACTERISTICS	
	Results Available Immediately	Results Available Later
Symptoms of central nervous system irritation	Pleocytosis—chiefly lymphocytes—30 to 3000 cells	Protein normal or elevated
Signs of nuchal-spinal rigidity	No organisms on smears (gram and acid-fast)	Chloride normal
No fixed neurologic signs or abnormal reflexes	Sugar level normal or elevated	All cultures negative
No pathognomonic signs elsewhere, viz., paresis, parotitis, rash, jaundice, chest x-ray		

course unfolds, but in most cases no pathognomonic clinical or laboratory sign appears.

The pathognomonic signs which may clarify the etiology usually appear in the next few days if they do at all. These include (1) clinical evidence of mumps, viz., swelling of the parotid or submaxillary salivary glands; orchitis; pancreatitis suggested by persisting abdominal pain, vomiting, back pain and elevated serum or urinary amylase; (2) paralysis of lower motor neuron type with no sensory changes, thus indicative of poliomyelitis; (3) changes in reflexes, abnormal reflexes, sensory disturbances, disturbances of mood and affect, indicative of viral encephalitis (herpes simplex, equine and St. Louis encephalomyelitis, and the like); (4) reticuloendothelial swellings and a white blood cell count and heterophile agglutination significant for infectious mononucleosis; (5) chest x-ray changes and positive Mantoux test indicative of tuberculosis; (6) clinical evidence of leptospirosis, i.e., icterus or urinary findings of nephritis, or both; (7) an eruption characteristic of herpes zoster.

But more often than not these diagnostically helpful signs do not appear. The picture becomes one of improvement over the ensuing week or ten days with no residual findings except mild "stiffness" of the back and leg muscles.

ETIOLOGY

The central nervous system and its meninges may react in a unitary fashion to a wide variety of infectious and noninfectious insults. Table 2 outlines the diverse possibilities of these, the viral group being more common than all the other causes together. By utilizing currently available viral techniques approximately 50 per cent of cases of this syndrome can be diagnosed specifically.

Infectious Causes

Viral infections make up the largest etiologic group. Table 3 enumerates most of the *known* viral causes of the syndrome. Of these, nonglandular mumps and nonparalytic poliomyelitis frequently present diagnostic difficulties. Table 4 enumerates some guides which may be clinically useful in distinguishing these two conditions. When hospitalized, such children should be handled as though they have the communicability of mumps, but they should have the close neuromuscular examinations (current and six to eight weeks later) of a poliomyelitis patient.

Although the prognosis is excellent and the management merely expectant in both nonglandular mumps and nonparalytic poliomyelitis,

TABLE 2. *Clinical Conditions Which May Induce the Acute Aseptic Meningitis Syndrome*

AGENT	DISEASE
I. Infectious	
A. Viral	
a. Established viral origin	
Transmitted:	
Man to man:	
Poliomyelitis, mumps, herpes simplex, herpes zoster, Coxsackie B, primary atypical pneumonia, infectious hepatitis	
Rodent to man:	
Lymphocytic choriomeningitis	
Arthropod to man:	
Arthropod-borne encephalitides, including Eastern, Western equine and St. Louis	
b. Presumed viral origin	Infectious mononucleosis, infectious lymphocytosis, cat-scratch fever
B. Spirochetal	
1. Leptospira icterohaemorrhagiae, canicola, pomona	Leptospirosis
2. Treponema pallidum	Syphilis
C. Yeast	E.g., torulosis
D. Protozoal	Toxoplasmosis
E. Helminthic	Trichinosis
F. Bacterial	
1. M. tuberculosis	
2. Bacteria causing purulent meningitis	1. Very early meningitis 2. Meningitis modified by partial suppression of organisms by suboptimal antibiotic therapy
II. Postinfectious	Measles, rubella, varicella, mumps, vaccinia, variola
III. Noninfectious	
A. Meningeal reaction from neighboring lesions	Sinusitis, mastoiditis and other abscesses, infiltrates, hematomas, thromboses in or adjacent to the central nervous system
B. Toxins and irritants	
1. Systemic poisons	E.g., lead, arsenic, bacterial
2. Intrathecal injections	Serums, antibiotics, air, Lipiodol
C. Allergy	
1. Medications	Serum sickness, including due to antibiotics
2. Prophylactic injections	Rabies vaccine, pertussis vaccine
3. Other allergens (?)	Seen rarely in association with urticaria and arthralgias

TABLE 3. *Infectious Causes of Acute Aseptic Meningitis Syndrome: Conditions* without *Pathognomonic Signs or Clinical Laboratory Findings*

DISEASE	SUSPICION RAISED BY	DEFINITIVE DIAGNOSIS REQUIRES
Common:		
Nonparalytic poliomyelitis	Poliomyelitis epidemic and known exposure	Stool collection; acute and convalescent serum
Nonglandular mumps	Exposure in a known susceptible	Acute and convalescent serum
Uncommon:		
Bornholm disease (pleurodynia), Coxsackie B viruses	Current epidemic	Stool collection; acute and convalescent serum
Leptospirosis, without hepatitis or nephritis	Swimming or other immersion in waters fouled with animal urine	Acute and convalescent serum
Lymphocytic choriomeningitis virus	Fall-winter seasons; exposure to mice	Acute and convalescent serum
Arthropod-borne encephalitis: mild cases	Epidemic areas in warm months	Acute and convalescent serum
Herpes simplex without eruption or stomatitis	—	Acute and convalescent serum

it is well to have the patient's paired serums tested. If the child has indeed had mumps, it will spare anxiety and needless isolation, and so on, in later life should he become re-exposed to mumps.

Of the nonviral but infectious causes, the most significant are (1) incipient tuberculous meningitis and (2) purulent meningitis modified or suppressed by preceding inadequate antibiotic therapy; this occurs particularly with *H. influenzae* type B. Each patient must be individualized; thus close contact with a tuberculous adult, the presence of a

TABLE 4. *Suggestive Differential Features**

	NONGLANDULAR MUMPS	NONPARALYTIC POLIOMYELITIS
History:		
Local epidemic	+	+
Past history of		
1. **Paralytic poliomyelitis**	− or +	Usually −
2. **Glandular mumps**	−	− or +
Direct contact	Often known	Often uncertain
Sex ratio	Males 3:1	Unaffected
Examination:		
Muscle spasm	Mild	Marked
Head-drop sign	Uncommon	Common
Clinical laboratory tests:		
Pleocytosis	+ + to + + + +	+ to + + +
Serum amylase	May be elevated	Normal
White blood cell count	Normal	Normal to slight elevation

* Definitive differential diagnosis of nonglandular mumps *requires* serologic tests (complement fixation, antihemagglutination or neutralization) of the patient's acute and convalescent serums. The mumps skin test alone is unreliable.

suspicious chest radiograph and certainly a positive Mantoux test would weigh more heavily than the fact that the spinal fluid chemistry is normal and free of tubercle bacilli on direct smear.* A course of antituberculous therapy could be indicated on the basis of a *presumptive* diagnosis. The clinical course in the ensuing fortnight may be indicative of the proper etiology. When one is faced with a difficult immediate decision between the acute aseptic meningitis syndrome and a partially suppressed bacterial meningitis, the clinical history is of great value. If no antibacterial agents were given, the former diagnosis becomes more likely. In the latter diagnosis the cerebrospinal fluid sugar level may be depressed and the peripheral white blood cell count elevated. One may occasionally be forced into the unhappy decision of treating the patient for a week or so with a *presumptive* diagnosis of bacterial meningitis. In

* In this diagnostic quandary, bone marrow smears for acid-fast bacilli, subsequently cultured and identified as *M. tuberculosis*, have been of immediate help to me in several infants.

such instances a combination of penicillin and tetracycline in full dosage is preferred. By proper individualization of cases the promiscuous use of antibiotics for this syndrome can be avoided.

RECURRENCES AND "ALLERGIC ASEPTIC MENINGITIS"

Recurrences of this syndrome are rare in children, despite the multiplicity of causes. The yeast infection torulosis, which may induce relapsing or subacute aseptic meningitis in adults, has not been observed to do the same in children.

Table 2 refers to some allergic causes associated with therapeutic injections. In addition, two children with recurrent urticaria and hydrathrosis were observed to have recurrent pleocytosis, unexplained but perhaps due to allergy. In view of the occurrence of synovial and other serous membrane reactions in response to diverse hypersensitivity states, the occurrence of similar meningeal reactions may be expected occasionally. Generally speaking, the diagnosis of "allergy," like the diagnosis "a virus condition," may too readily become the refuge of the diagnostically destitute.

SPECIAL SEROLOGIC AND OTHER TESTS

The blood serum stored immediately upon suspicion of this diagnosis is paired with one taken ten to fourteen days later. Some state health department laboratories can do special serologic tests, and those laboratories without these facilities can forward the serum pair together with a concise clinical summary to: Virus and Rickettsia Section, Communicable Disease Center, Public Health Service, U.S. Dept. of Health, Education and Welfare, P. O. Box 61, Montgomery 1, Alabama.

The tests generally conducted for the aseptic meningitis syndrome include those for showing antibody rise against Eastern and Western equine encephalitis, St. Louis encephalitis, lymphocytic choriomeningitis, mumps, psittacosis and lymphogranuloma group viruses and leptospirosis.

Under special circumstances and arrangements stools collected in the acute stage may be tested for the presence of poliomyelitis virus and for the Coxsackie group of viruses. The giant strides being made in tissue culture studies of these viruses will soon bring diagnostic aid to the clinician in this field. It is seldom that cerebrospinal fluid specimens are used in practice for virus isolations, although mumps, herpes simplex, Coxsackie B and lymphocytic choriomeningitis viruses may be recovered occasionally by inoculating suckling and adult mice or chick embryos.

MANAGEMENT AND PROGNOSIS

Provided close clinical observation reveals that the child fits clearly into the category summarized in Table 1, the management is expectant, the prognosis excellent. If such a child is hospitalized during a large poliomyelitis outbreak, he should be kept isolated from the others unless it is certain that he has already had mumps in the past. In other words, the isolation required for mumps should be applicable to all children with this syndrome until a reasonably confident clinical diagnosis to the contrary is made.

Instances requiring the use of antibiotics become rare as one's familiarity with this syndrome increases. Extenuating circumstances in this regard are mentioned under infectious nonviral causes of the syndrome.

The nuchal-spinal rigidity, the fever and headache may require respectively the use of hot packs and gentle physical therapy, cool sponging, salicylates and barbiturate sedation. Vomiting and abstention from food and fluid intake for several days may require short-term parenteral alimentation.

CONCLUSION

The causes of this syndrome are many. To label a child as belonging to this group requires an excellent history often probing subtle points concerning exposures, and keen clinical evaluation for fine points in the physical examination. Sterile blood serum collected when the child is first seen and again ten to fourteen days later when suitably tested may elucidate the etiologic diagnosis in as many as 50 per cent of cases. When hospitalized, such children should be regarded as contagious as those with mumps, but should be closely examined currently and six to eight weeks later for minor evidences of lower motor neuron paralysis. There is a growing awareness of this syndrome and a better understanding of its multiple etiology.

REFERENCES

1. Adair, C. V., Gauld, R. L., and Smadel, J. E.: Aseptic Meningitis, a Disease of Diverse Etiology. Ann. Int. Med., 39:675, 1953.
2. Scott, T. F. McN., and Steigman, A. J.: The Acute Aseptic Meningitis Syndrome, in Nelson, W. E.: Textbook of Pediatrics. 6th ed. Philadelphia, W. B. Saunders Company, 1954.
3. Wallgren, A.: Une nouvelle maladie infectueuse au système nerveux central? Acta paediat., 4:158, 1925.

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RABIES

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Rabies is an acute infectious disease characterized by a profound dysfunction of the central nervous system.

HISTORY

Mankind has been familiar with the disease—also more descriptively named hydrophobia or lyssa—since time immemorial. Democritus and Aristotle, and possibly Homer, knew it as a disease of animals,[1] whereas Celsus was the first to describe its transmissibility to man. Its existence in the Western Hemisphere has been recognized since 1709, and in North America since 1753. The modern era of research in the field of rabies dates from the time of Pasteur[10] and his associates, who were able to transmit the causative agent to laboratory animals and to modify its pathogenicity by serial intracerebral passages in these hosts.

ETIOLOGY

The disease is caused by a virus which, having a diameter of 100 to 150 millimicrons,[5] can be filtered through bacteria-retaining filters. The virus is readily inactivated by formalin, bichloride of mercury, strong acids and certain detergents. The virus is resistant to the action of phenol, but can be inactivated in an aqueous solution if kept for an hour at 56° C. The infectious agent can best be preserved either in the frozen or desiccated state or in a solution containing equal parts of glycerin and buffered saline.

CHARACTERISTICS OF THE INFECTIOUS AGENT

There is only one immunologic type of rabies virus: i.e., all the strains isolated so far throughout the world are serologically identical. The term "street virus" is applied to the agent causing the disease in

nature, which is characterized by variable, usually long, incubation periods, and by the presence of the virus in salivary glands as well as in the central nervous system of the infected host. In contrast to "street virus," the term "fixed virus" refers to a strain of rabies adapted to laboratory animals or developing chick embryos by numerous serial passages. Infection of laboratory animals with fixed virus is characterized by a short (usually four to six days) incubation period and by the apparent inability of the agent to multiply in the salivary gland tissue of the host.

HOST RANGE

The disease can affect all mammals, including bats. Birds are also susceptible to infection with rabies virus, although less so than mammals. Among the domestic animals, in addition to dogs and cats, cattle are particularly susceptible.[6] In the laboratory, hamsters, mice, guinea pigs and rabbits, in the order of decreasing susceptibility, are used for the establishment of experimental infection.

GEOGRAPHIC DISTRIBUTION; VECTORS; SOURCE OF INFECTION

Figure 8 presents the geographic distribution of rabies in relation to the more common vectors of the disease. With the exception of Australia, the British Isles and some Scandinavian countries, rabies extends throughout the world. From the epidemiologic point of view, Johnson[5] separates the disease into two categories: the natural, sylvatic type present in enzootic form in wildlife—wolves, mongooses, bats, foxes, coyotes, skunks, and so on; and the urban type, affecting domestic animals. Among the latter, dogs are mainly responsible for human infection. Once canine rabies is eliminated, there is no evidence that rabies persists in urban areas. The disease is not transmitted by insects or arthropods.

Because of the existence of the wildlife reservoir, the actual incidence of rabies in animals in any given geographic area is unknown. Seven to 10,000 cases of rabies in animals are usually reported each year[12] in the United States, but it is certain that the true figure is much higher.

INCIDENCE

The incidence of rabies in man, who becomes an accidental host through exposure to the saliva of a rabid animal, varies from country to country. There are no more than twenty human cases reported annually in the United States, but the number of human exposures to the bites of animals either proved to be rabid or of undetermined status

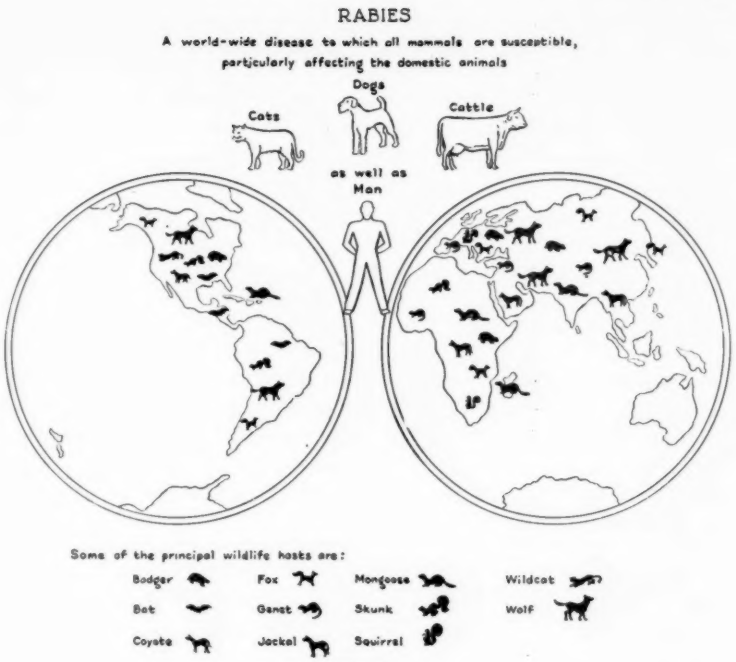

Fig. 8.

runs into tens of thousands. The incidence of rabies in children is high for several reasons. Many wild animals lose their fear of man when rabid, and since children's approach to animals is generally more friendly than adults', the chance of exposure is increased. It is also true that a child is more defenseless than an adult when confronted with an animal suffering from the furious type of the disease. It seems, therefore, desirable during a severe epizootic to prevent children from roaming freely, or even to keep them inside unless accompanied by an adult.

ENTRY OF VIRUS INTO THE HOST; ATTACK RATE

The virus enters the body from the saliva of the infected animal through a wound or scratch on the skin, usually inflicted by the animal. Infection through unabraded mucosa also seems possible.[9] The virus may remain arrested in viable form at the site of infection for some time before spreading via nerve trunks to the central nervous system, where it multiplies. It may then spread centrifugally, infecting and possibly multiplying in the salivary glands. There is strong evidence against

the possibility that blood plays any role in the dissemination of the virus throughout the body.[14] Rabies virus cannot invade the body through an intact gastrointestinal tract.

The attack rate in man following bites of rabid animals depends on the severity of the lacerations and the amount of virus introduced into the wounds. Neck and head bites lead to a higher incidence of infection, probably because of the greater severity of the actual lacerations. Recent data[4] indicate a 47 per cent attack (and mortality) rate in thirty-two persons who were bitten by the same rabid wolf and who received no postexposure treatment.

PATHOLOGY

The changes resulting from rabies virus infection revealed at autopsy are located mainly in the central nervous system. The brain is edematous and congested, with broad and flattened convolutions. The blood vessels of the brain, medulla and spinal cord are usually congested, in the white and gray matter alike. If salivary glands are infected with virus, they are soft and swollen.[2]

The microscopic findings are nonspecific, indicating an acute encephalitis evidenced by hyperemia, perivascular and perineuronal infiltration with mononuclear cells, and marked neuronal degeneration. Changes revealed by microscopic examination of virus-infected salivary glands (parotid, sublingual and submaxillary) may consist in mononuclear cell infiltration of periacinal interstitial tissue in the presence of degeneration of acinar cells.[2]

Intracytoplasmic inclusion bodies in the nerve cells which become cherry red when touch-impression slides of brain tissue are treated with Seller's stain are pathognomonic for rabies. In the absence of these so-called Negri bodies the lesions cannot be distinguished from those observed in other viral encephalitides. Some cases occur, however, in which Negri bodies are not demonstrable and yet rabies virus is isolated by animal inoculation.[17]

INCUBATION PERIOD

The incubation period of rabies ranges from ten days to twelve months, although it is usually from fifteen days to five months.[5] The amount of virus introduced into the wound and the severity of the laceration influence the length of the incubation period, without direct relationship to the site of the original exposure.

It is imperative in taking a history that questions be asked family associates—since the patient himself may be disoriented and often even unconscious—about incidents occurring weeks before the onset of illness.

Even if a bite cannot be remembered, questions should be asked about the behavior of animals with which the patient has had contact. Often, minor and seemingly insignificant contacts with an animal not obviously sick at the time of exposure are forgotten by both the patient and his family. Specific inquiry should be made as to the origin of any scar tissue on the body observed in the course of examination. There is always the possibility of exposure to rabies in the weeks or months preceding an encephalitis of unknown etiology.

CLINICAL PICTURE AND DIAGNOSIS

The clinical picture is essentially that of an encephalitis. The prodromal phase is often marked by fever, malaise, nausea and sore throat. In infants, fever, refusal of food, irritability, restlessness and disorientation have been described[2, 5] as characteristic of the prodromal stage. Abnormal sensations around the site of infection, such as intermittent pain, tingling or burning, are of diagnostic significance. In some cases persistent loose cough may also manifest the disease.[2] Hyperesthesia of the skin to temperature changes and to drafts may be caused by the extreme stimulation of the general sensory system. Sometimes increased muscular tonus, dilation of pupils and increased salivation are present.

As the disease progresses, almost always accompanied by high body temperature, the child becomes more distraught, apprehensive and restless. Periods of hyperexcitability may alternate with periods of lethargy. Spasmodic muscular contractions, often affecting the respiratory muscles and resulting in labored breathing, sometimes occur in the hyperexcitability periods. Spasmodic contractions of the muscles of the mouth, pharynx and larynx on drinking—and often at the mere sight of fluid—are observed in many, though by no means all, cases. This dysfunction in deglutition, which leads the patient to gag and tremble when offered fluid, gave the disease its common name—hydrophobia, or fear of water.

As the illness develops further, the pulse becomes rapid and the pupils dilate and remain fixed. There is usually general hyporeflexia. Periods of irrational and even maniacal behavior are interspersed less and less frequently with those of alertness and responsiveness. Complete paralysis of the muscles of phonation may lead to a loss of voice. The excitation phase of the disease may give way, shortly before death, to cessation of muscle spasm, peripheral vascular collapse and general paralysis of the flaccid type.

In some cases, particularly those observed in Trinidad, B.W.I.,[11] after vampire bat bites, the clinical course of rabies is characterized almost exclusively by the syndrome of ascending paralysis without excitation or hydrophobia.

Clinical laboratory findings are, in general, uninformative.

TABLE 5. *Indications for Specific Postexposure Treatment*

NATURE OF EXPOSURE	CONDITION OF ANIMAL		RECOMMENDED TREATMENT
	At Time of Exposure	During Observation Period of 10 Days	
I. No lesions Indirect contact only	Rabid	—	None*
II. Licks			
1. **Unabraded skin**	Rabid	—	None*
2. **Abraded skin and abraded or unabraded mucosa**	(a) Healthy	Healthy	None
	(b) Healthy	Clinical signs of rabies or proved rabid	Start vaccine at first signs of rabies in anima
	(c) Signs suggestive of rabies	Healthy	Start vaccine immediately. Stop treatment if animal is normal on 5th day after exposure †
	(d) Rabid, escaped, killed or unknown	—	Start vaccine immediately
III. Bites			
1. **Simple exposure**	(a) Healthy	Healthy	None
	(b) Healthy	Clinical signs of rabies or proved rabid	Start vaccine at first signs of rabies in animal
	(c) Signs suggestive of rabies	Healthy	Start vaccine immediately. Stop treatment if animal is normal on 5th day after exposure †
	(d) Rabid, escaped, killed or unknown; or any bite by wolf, jackal, fox or other wild animal	—	Start vaccine immediately
2. **Severe exposure (multiple; or face, head or neck bites)**	(a) Healthy	Healthy	Hyperimmune serum immediately. No vaccine as long as animal remains normal
	(b) Healthy	Clinical signs of rabies or proved rabid	As in III, 2, (a), but start vaccine at first sign of rabies
	(c) Signs suggestive of rabies	Healthy	Hyperimmune serum immediately, followed by vaccine. Vaccine may be stopped if animal is normal on 5th day after exposure
	(d) Rabid, escaped, killed or unknown. Any bite by wild animal	—	Hyperimmune serum immediately, followed by vaccine

Hyperimmune serum to be effective must be given within 72 hours of exposure.
These indications apply equally well whether or not the biting animal has been previously vaccinated.
* Start vaccine immediately in young children and patients when a reliable history cannot be obtained.
† Alternative treatment would be to give hyperimmune serum and not start vaccine as long as animal remained normal.

The clinical diagnosis of the disease can be based on profound dysfunction of the central nervous system accompanied by impairment in deglutition, following a history of contact with an animal several weeks or months prior to the onset of illness. Diagnosis can be confirmed by the isolation of virus identified as rabies from saliva obtained in the course of the disease[3] or from brain and salivary gland tissue[2] obtained at autopsy and studied in the laboratory.

TREATMENT*

Strictly speaking, the term "treatment" cannot be applied to rabies, since the disease, once clinically apparent, is invariably fatal. Measures recommended here are to be applied immediately after exposure to injury by an animal and prior to the development of any signs and symptoms of the disease.

All bite wounds, including skin abrasions exposed to licks of animals, should be cleansed immediately with soap or detergent solutions. Zephiran chloride in a 1 per cent solution has proved effective in laboratory animals.[13] Puncture wounds not easily reached by these agents may be treated with strong mineral acids.

The commonly used antibiotics have no beneficial effect, except in combating any bacterial infection which may be present at the site of a wound.

A summary of indications for specific postexposure treatment has recently been published (Table 5) by the Expert Committee on Rabies of the World Health Organization.[16] The pediatrician is often handicapped by the difficulty of getting an accurate and reliable description of a possible exposure from a child, and this has to be taken into consideration in evaluating the degree of exposure before instituting treatment.

If a bite is inflicted by a domestic animal—viz., dog, cat, and the like—the physician should advise that the animal be kept alive, but that it be restrained immediately and placed under proper surveillance. The diagnosis of rabies in the living animal should be made by a qualified practitioner of veterinary medicine or veterinary public health officer. Frequent reports on the clinical status of the animal must be supplied to the physician, since the course adopted in the treatment of the patient is directly influenced thereby (Table 5). Postmortem diagnosis in animals should also be conducted by trained workers in a properly equipped laboratory.[17]

* In a rabies endemic area and adjacent territory, any wound inflicted on a human being by an animal is an imperative indication for treatment until the animal is proved to be nonrabid, either by clinical observations or by the absence of rabies virus from tissues obtained after death.

Passive immunization against rabies by administration of hyperimmune antiserum is recommended by the Expert Committee as standard procedure in view of overwhelming experimental evidence in favor of such treatment. Experience in animals indicates that antirabies serum is most effective when administered within seventy-two hours after exposure.[7, 8] In initiating treatment, however, more than seventy-two hours after exposure, antirabies serum followed by vaccine should be more effective than would vaccine alone. Various vaccines exist, and any which meets the standard potency requirement may be used.

No rabies vaccine preparation is at present available which is free from nervous tissue, the presence of which may give rise to local allergic reactions or even to those of a general neuroparalytic nature. Vaccine treatment should be interrupted immediately at the slightest sign of nervous system impairment. Also, vaccine treatment should not be given unless specifically indicated (see Table 5).

RECENT ADVANCES IN CONTROL OF THE DISEASE

Rabies is, in a sense, a preventable disease. However, the low incidence of infection does not warrant the prophylactic immunization of man as a control measure. Human exposures can be averted by the effective control of the disease in dogs and other animals.

The most efficient method of combating canine rabies in a given area is a mass immunization program aiming at the vaccination of at least 70 per cent of the entire dog population.[16] The use of a vaccine conferring long-lasting immunity makes such a program easier to execute. The mass vaccination program should be accompanied by the registration and restraint of dogs and by the elimination of stray animals. At the same time, measures should be initiated to reduce wildlife populations of species known to be a threat in a particular section. The rabies enzootics in bats present the most formidable problem of control, although some progress has been made recently in the prevention of human contacts with infected bats in Trinidad, B.W.I.

None of these measures will be fully effective until there is widespread understanding of the rabies problem by professional workers and the lay public alike and until there is international cooperation in solving it. Much progress in this direction has been made in recent years,[15, 16] and it is hoped that this disease—so feared by everybody, particularly parents—will come under effective control.

REFERENCES

1. Babes, V.: Traité de la rage. Paris, Librairie J.-B Baillière et Fils, 1912.
2. Bernsten, C. A., Jr., and Stevenson, L. D.: Human Rabies. A Case Report with Necropsy Studies Wherein Brain and Salivary Gland Yielded Virus: Notes

on the Viral Tissue Distribution. J. Neuropath. & Exper. Neurol., *12*:169, 1953.
3. Duffy, C. E., Woolley, P. V., Jr., and Nolting, W. S.: Rabies. A Case Report with Notes on the Isolation of the Virus from Saliva. J. Pediat., *31*:1, 1947.
4. Gremliza, L.: Kasuistik zum Lyssa-Problem. Ztschr. Tropenmed. u. Parasitol., *4*: 382, 1953.
5. Johnson, H. N.: Rabies, in Rivers, T. M.: Viral and Rickettsial Infections of Man. Philadelphia, J. B. Lippincott Company, 1952, pp. 267–299.
6. Koprowski, H., and Black, J.: Studies on Chick-Embryo-Adapted Rabies Virus. VII. Immunological Responses of Animals to Vaccination with High Egg Passage Flury Strain. J. Immunol., *72*:503, 1954.
7. Koprowski, H., and Cox, H. R.: Recent Developments in the Prophylaxis of Rabies. Am. J. Pub. Health, *41*:1483, 1951.
8. Koprowski, H., Van der Scheer, C. E., and Black, J.: Use of Hyperimmune Anti-rabies Serum Concentrates in Experimental Rabies. Am. J. Med., *8*:412, 1950.
9. Leach, C. N., and Johnson, H. N.: Human Rabies, with Special Reference to Virus Distribution and Titer. Am. J. Trop. Med., *20*:335, 1940.
10. Pasteur, L.: Lettre sur la rage. Ann. Inst. Pasteur, *1*:1, 1887.
11. Pawan, J. L.: The Transmission of Paralytic Rabies in Trinidad by the Vampire Bat (*Desmodus Rotundus Murinus* Wagner, 1840). Ann. Trop. Med. *30*: 101, 1936.
12. Proceedings of the United States Livestock Sanitary Association, 57th Annual Meeting, 1953, p. 314.
13. Shaughnessy, H. J., and Zichis, J.: Treatment of Wounds Inflicted by Rabid Animals. Bull. World Health Organization, *10*:805, 1954.
14. Schweinburg, F.: Neuere Ergebnisse der Tollwutforschung. Ergeb. Hyg. Bakteriol. Immunitätsforsch. u. Exp. Therap., *20*:1, 1937.
15. World Health Organization. Expert Committee on Rabies. Report on the First Session. Technical Report Series No. 28, Geneva, 1950.
16. World Health Organization. Expert Committee on Rabies. Second Report. Technical Report Series No. 82, Geneva, 1954.
17. World Health Organization. Laboratory Techniques in Rabies. Monograph Series No. 23, Geneva, 1954.

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Q FEVER AND PSITTACOSIS

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Q FEVER

Q ("QUERY") fever, first described in Australia by Derrick,[8] is an infection due to *Coxiella burneti*, characterized by fever, headache, malaise, and frequently by pulmonary involvement.

ETIOLOGY

Coxiella burneti (Rickettsia burneti, Rickettsia diaporica) differs from other rickettsiae in that it passes ordinary filters, produces no soluble antigens, and does not cause Proteus agglutinins to appear in the blood of infected persons. Like other rickettsiae, it is an obligate intracellular parasite. Its high degree of resistance to desiccation presumably plays an important role in the transmission of Q fever. Experimental infection[15] may be produced by inoculation into mice, hamsters, guinea pigs or chick embryos. Guinea pigs, the animals used most commonly for isolation of the infectious agent, acquire a febrile illness to which they frequently succumb. Splenomegaly is the most striking postmortem finding. After intraperitoneal inoculation some exudate may be found on the abdominal organs. The rickettsiae may be demonstrated by Machiavello's method as a red-staining lanceolate or bipolar rod or as a diplobacillus. Inoculation of five or six day old chick embryos via the yolk sac is usually followed by death after incubation for four or five days. Antigens for diagnostic tests, such as agglutination or comple-

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ment fixation reactions, may be prepared from infected chick embryo material.

EPIDEMIOLOGY

Q fever is of widespread distribution. It occurs in North America, Central America, England, the Mediterranean area of Europe and Asia, and in Africa and Australia.[15] It is not a common clinical infection in any age group, and is particularly uncommon in children. In 180 cases described by Clark et al.[6] none of the patients was less than nine years old, and only eleven were between ten and nineteen years of age. The disease often appears in epidemic form in certain areas. In such areas, in addition to clinically recognized cases, a significant proportion of the population manifests circulating antibodies against the disease.[2] In Australia it exists as an enzootic disease in bandicoots and other wild animals. In the United States the rickettsia has been recovered from cattle, sheep and goats.[2, 11] The organism has been recovered from raw milk of cows and sheep, as well as from placentas and feces of these animals. Although ticks (*Dermacentor andersoni, Amblyomma americanum*) naturally harbor *Coxiella burneti*,[7] tick bites are probably an uncommon source of infection. In some outbreaks the drinking of raw milk was commonly associated with the disease;[2] in other outbreaks environmental contact with sheep, goats or cattle or their products (hides, fertilizer, milk) has appeared to be a frequent factor.[11] In the Northern California outbreak inhalation of infected dust apparently was the usual source of infection.[11] Man-to-man spread of Q fever occurs extremely rarely, if at all, although laboratory infections have been reported.[12]

PATHOLOGY

Q fever is rarely fatal, so that pathologic information is scant. In one fatal case[12] congestion and edema of the lungs were observed. Histologic examination of patchy consolidated areas in the lung revealed a fibrinocellular exudate in the alveoli, consisting principally of lymphocytes, plasma cells, large mononuclear cells and erythrocytes. The bronchial epithelium was desquamated. Hyperplasia of the alveolar epithelium and thickening of the interalveolar septa were noted.

CLINICAL PICTURE

The incubation period ranges from fourteen to twenty-six days. The average incubation period is a little less than three weeks. In about three quarters of patients the onset is abrupt. All recognized cases are

febrile, with temperatures sometimes as high as 105° F. Chills occur in three out of four patients and are often severe. Sometimes two rigors occur in the same day. Severe headache is a common complaint and should raise suspicion of Q fever. Muscle and joint pains are also frequent, and are similar to those found in many other febrile diseases. In spite of relatively frequent pulmonary involvement, cough, usually dry, rarely productive of bloody sputum, occurs in only one quarter of patients. Symptoms referable to the upper respiratory tract are sufficiently less frequent to be of negative diagnostic value.[6]

Positive physical findings are not generally observed. Rales, generally transient, may be heard in one fifth of patients[6] and are rarely associated with other signs of consolidation. Hepatomegaly was observed in 11 per cent of patients in one outbreak[6] and was sometimes associated with jaundice and altered liver function tests. Such patients may be thought to have infectious or amebic hepatitis. Rashes are rarely seen, and no typical eruption has been described.

LABORATORY DIAGNOSIS

In most patients the white blood cell count is low or normal early in the disease; leukocytosis often appears late in the course. The incidence of pulmonary infiltration demonstrated radiologically is variable. In the group reported by Clark et al.[6] only one third demonstrated such x-ray abnormality.

The diagnosis depends either on isolation of the etiologic agent by animal inoculation with the patient's blood or on demonstration of a rising titer of complement-fixing or agglutinating antibodies during the course of the disease and early convalescence. Agglutinating antibodies usually are not found before the ninth day of illness. By the end of the fourth week they are found in 90 per cent of patients. Complement-fixing antibodies appear during the second week of the disease and reach a peak about the end of the third week. Since appreciable antibody titers may persist for long periods, even after unapparent infection, comparison of the titer of an "acute" blood specimen obtained during the early stages of illness with a "convalescent" blood specimen obtained a week or more later is essential for accurate diagnosis.

COURSE

The course of Q fever is variable and is influenced to a striking degree by the age of the patient. Most children and young adults suffer from an illness resembling influenza or primary atypical pneumonia lasting three to seven days, rarely as long as two weeks. On the other hand, adults over forty years old often remain ill for many weeks with an ill-

ness that may suggest brucellosis, hepatitis, typhoid fever, psittacosis or coccidioidomycosis. The fever, which is usually of abrupt onset, is generally of swinging character during the fastigium, and then declines by rapid or slow lysis. Complications are rare in young persons. Thrombophlebitis occurs occasionally in older patients.[6]

TREATMENT

Rest in bed is indicated during the febrile phase and for a short time thereafter. Aspirin usually ameliorates the headache and myalgia. The cough only occasionally is sufficiently severe to require sedation. Attention should be paid to adequate caloric, protein and vitamin intake in protracted cases.

Chemotherapeutic treatment is less successful in Q fever than in other rickettsial diseases. Chlortetracycline may be given in dosage of 40 mg. per kilogram per day in four daily doses.[5] Oxytetracycline[3] and chloramphenicol[10] have been used successfully in a few cases. Presumably, tetracycline will prove similarly effective. Penicillin, streptomycin and sulfonamides are of no value. Treatment should be continued until the patient has been afebrile for three or four days, since relapse may occur in insufficiently treated patients.

PSITTACOSIS

Psittacosis (ornithosis) is a generalized infection, usually with pulmonary involvement, due to a virus acquired by man from birds.

ETIOLOGY

The virus of psittacosis is one of a closely related group which includes that of lymphopathia venereum, meningopneumonitis and several other viruses.[13] The elementary bodies of psittacosis invade cells and produce a common matrix which later disperses to two or more foci in the cytoplasm. These large inclusion bodies may be 2 to 12 microns in diameter. At this stage the elementary bodies stain red with Machiavello's method, in contrast to the blue of the matrix. Multiplication then produces many small elementary bodies, eventually followed by death and rupture of the cell. Psittacosis virus may be cultivated in tissue cultures and chick embryos. Mice, hamsters, cotton-rats, monkeys and a wide variety of birds may be infected experimentally.

EPIDEMIOLOGY

Psittacosis is of widespread geographic distribution. The epidemic of 1929-30 involved 750 to 800 persons in twelve countries.[13] All ages and

both sexes are subject to the disease, but clinical infection is rare in children. Armstrong[1] reported that only eight of 169 patients were less than fourteen years old, and only one was less than four. In the British outbreak[16] five of seventy-nine patients were less than fourteen years old. The youngest of these was seven. None of Meyer's 111 patients was less than nine years old.[14] He stated that subclinical infection was not rare in older children.

Though man-to-man spread of psittacosis occurs occasionally, almost all cases arise from contact with infected birds. Often the avian source is an apparently healthy bird which sheds the virus in his droppings. Parrots, parakeets and pigeons are the most common sources of infection, but many other birds, including chickens, ducks, turkeys, canaries and pheasants, have been incriminated.

PATHOLOGY

The principal pathologic finding[13] in fatal cases is a patchy bronchopneumonia. The alveoli contain fibrin, lymphocytes, macrophages and desquamated alveolar epithelial cells. Active phagocytosis is evident, and intracytoplasmic inclusion bodies may be seen. Polymorphonuclear leukocytes are typically absent from the exudate. The relative lack of bronchitis and bronchiolitis is characteristic but not unique, and may serve to distinguish psittacosis from other viral pneumonia. The liver is usually congested and exhibits focal necrosis. The Kupffer cells contain elementary bodies. In addition, toxic changes may be observed in the heart, kidneys, adrenals and central nervous system.

CLINICAL PICTURE

The incubation period of psittacosis ranges from one to two weeks. The average period is ten days. History of contact with birds is usually elicited, but is absent in a significant number of patients. Though the bird may be ill, more often it is in apparent good health. Frequently the bird has been recently acquired. The infection is usually heralded by chilly sensations followed by a gradual rise of temperature. Anorexia, photophobia and headache are common early symptoms. Epistaxis occurs in one fourth of patients and may lead to suspicion of typhoid fever. A dry cough is usually noted, but is often not severe, especially in the early stages. Hemoptysis is rare, as is pleurisy. One is usually impressed principally with the toxic appearance of the patient, particularly in late cases. This observation, together with relative bradycardia and the occasional occurrence of splenomegaly and rose spots, may suggest typhoid fever to the clinician. In early cases the physical findings of pneumonia are usually lacking, but suppressed and altered breath

sounds and sticky rales, sometimes accompanied by dullness to percussion and bronchophony, may be noted in later cases. Delirium is fairly frequent. Either diarrhea or constipation may be present. In severely ill patients circulatory collapse manifested by tachycardia, dyspnea, cyanosis and hypotension may occur.

LABORATORY DIAGNOSIS

The white blood cell count is usually not altered dramatically. Leukopenia occurs in about one quarter of patients. A high percentage of nonfilamented leukocytes may be observed. Leukocytosis may occur late in the course. Albuminuria, probably of toxic origin, may be found, and occasionally glycosuria occurs. Even in the early stages when no abnormal physical findings are to be found in the chest, pulmonary infiltration is usually demonstrable radiographically.

The diagnosis must be confirmed either by isolation of the etiologic agent by animal inoculation or by demonstration of a rise of titer of circulating antibodies in serial blood specimens. Citrated blood or sputum may be inoculated into mice for virus isolation. Viremia may last as long as two weeks, and virus has been recovered from the sputum as late as the twenty-sixth day of illness.[13] Complement-fixing antibodies may begin to appear during the first week of disease, rise in titer for several weeks, and then persist for months. Accordingly, a rise in antibody titer between an "acute" blood specimen and a "convalescent" blood specimen obtained a week or more later is necessary for exact diagnosis. Early chemotherapeutic treatment may suppress or delay the appearance of complement-fixing antibodies. Patients with lymphopathia venereum often have significantly high antibodies against psittacosis virus,[13] but these usually decline rapidly in titer in contrast to those appearing during the course of psittacosis. Occasionally, anamnestic reactions may appear in patients with Q fever or brucellosis.

COURSE

In children psittacosis may be a subclinical or mild infection, as it may occasionally be in adults. The fever in mild cases declines after a week or ten days; in more severe infections it may persist for three or more weeks. In untreated patients the pneumonia may be progressive or migratory. Apathy or irritability to the point of delirium occurs frequently, especially in elderly persons. Thrombophlebitis is a common complication and may result in pulmonary embolization. Relapses are common in untreated patients and even in patients treated for an insufficient period with antibiotics. Convalescence may be prolonged in severe cases.

TREATMENT

Bed rest should be enforced until sufficiently long after defervescence to allow the patient to regain strength. Thrombophlebitis should be anticipated and avoided, if possible, by leg exercises and by wrapping of the lower extremities if necessary. Adequate caloric, vitamin and protein content of the diet should be ensured. Restlessness may require sedation with small doses of phenobarbital. Sedative cough mixtures may be necessary to suppress exhausting coughing. In the event of collapse the administration of plasma and cortisone must be considered.

Though penicillin in large doses (1,000,000 units daily)[9] is generally effective, small doses of repository-type penicillin may not result in improvement.[4] Chlortetracycline has proved to be highly effective when administered in a dose of 40 mg. per kilogram per day in four divided doses.[4] Initial therapy may be by the intravenous route. Oxytetracycline has been used in fewer patients, but presumably is similarly effective. Tetracycline probably will prove to be as effective as its derivatives. Chloramphenicol is thought to be inferior to drugs of the tetracycline group.[17] Since chemotherapy is largely suppressive, prolonged treatment is essential to prevent relapse. Antibiotics should be continued for a minimum of seven and preferably ten days, in spite of rapid initial favorable response. In untreated cases the mortality rate approximates 10 per cent. In patients properly treated with antibiotics this has been sharply reduced, so that a fatal outcome need rarely be expected, except in neglected cases or elderly debilitated patients.

REFERENCES

1. Armstrong, C.: Psittacosis: Epidemiological Considerations, with Reference to the 1929–1930 Outbreak in the United States. Pub. Health Rep., *45*:2013, 1930.
2. Bell, J. A., Beck, M. D., and Huebner, R. J.: Epidemiologic Studies of Q Fever in Southern California. J.A.M.A., *142*:868, 1950.
3. Bickel, G., and Plattner, H.: La Terramycine en thérapeutique. Schweiz. med. Wchnschr., *81*:1, 1951.
4. Brainerd, H., Lennette, E. H., Meiklejohn, G., Bruyn, H. B., and Clark, W. H.: The Clinical Evaluation of Aureomycin. J. Clin. Investigation, *28*:992, 1949.
5. Clark, W. H., Lennette, E. H., and Meiklejohn, G.: Q Fever in California. III. Aureomycin in the Therapy of Q Fever. Arch. Int. Med., *87*:204, 1951.
6. Clark, W. H., Lennette, E. H., Railsback, O. C., and Romer, M. S.: Q Fever in California. VII. Clinical Features of 180 Cases. Arch. Int. Med., *88*:155, 1951.
7. Davis, G. E., and Cox, H. R.: A Filter-Passing Infectious Agent Isolated from Ticks. I. Isolation from *Dermacentor Andersoni*, Reactions in Animals, and Filtration Experiments. Pub. Health Rep., *53*:2259, 1938.
8. Derrick, E. H.: Q Fever, a New Fever Entity: Clinical Features, Diagnosis, and Laboratory Investigation. M. J. Australia, *2*:281, 1939.
9. Goggio, A. F.: Human Psittacosis Cured by Penicillin Therapy. Calif. Med., *167*: 70, 1949.

10. Harman, J. B.: Q Fever in Great Britain. Clinical Account of Eight Cases. Lancet, 2:1028, 1949.
11. Lennette, E. H., and Clark, W. H.: Observations on the Epidemiology of Q Fever in Northern California. J.A.M.A., *145*:306, 1951.
12. Lillie, R. D., Perrin, T. L., and Armstrong, C.: An Institutional Outbreak of Pneumonitis. III. Histopathology in Man and Rhesus Monkeys in the Pneumonitis Due to the Virus of Q Fever. Pub. Health Rep., *56*:149, 1941.
13. Meyer, K. F.: Psittacosis-Lymphogranuloma Group, in Rivers, T. M., ed.: Viral and Rickettsial Infections of Man. 2d ed. Philadelphia, J. B. Lippincott Company, 1952, pp. 440–464.
14. Meyer, K. F., and Eddie, B.: The Knowledge of Human Virus Infections of Animal Origin. J.A.M.A., 133:822, 1947.
15. Smadel, J. E.: Q Fever, in Rivers, T. H., ed.: Viral and Rickettsial Infections of Man. 2d ed. Philadelphia, J. B. Lippincott Company, 1952, pp. 652–664.
16. Sturdee, E. L., and Scott, W. M.: A Disease in Parrots Communicable to Man (Psittacosis). Ministry of Health Rep. No. 61. London, His Majesty's Stationery Office, 1930.
17. Wells, E. B., and Finland, M.: Comparative Effect of Aureomycin and Chloromycetin on Psittacosis Infection in Chick Embryos. Proc. Soc. Exper. Biol. & Med., 72:365, 1949.

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BRUCELLOSIS IN CHILDREN

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Brucellosis is primarily an infectious disease of domestic animals—the cow, hog, goat and sheep—transmissible to man. The etiologic agent is a nonmotile, nonsporulating, gram-negative bacillus or coccobacillus. There are three recognized species of the organism, *Brucella melitensis, Br. abortus* and *Br. suis. Brucella melitensis (Micrococcus melitensis)* was the first of these shown to cause disease in man. Bruce,[9] in 1886, found the organism in autopsy material from a patient dying of Malta fever (Mediterranean fever). Later, Bruce[10] and Hughes,[32] who coined the term "undulant fever," firmly established the organism as the causative agent of the disease. The role of the goat as the reservoir of the infection and its transmission through milk were not recognized until 1905.[63] *Brucella abortus,* the etiologic agent of infectious abortion in cattle, was discovered by Bang in 1897.[4] *Brucella suis* was isolated from aborting swine by Traum in 1914.[61] Human disease due to these latter two species was not recognized until much later. Although the relationship between Bang's bacillus and the causative agent of Malta fever was demonstrated by Alice Evans[18] in 1918, it was not until 1924 that the first case of brucellosis due to a species other than *Br. melitensis* was reported.[36] After this the knowledge of brucellosis in both animals and man due to all three species grew rapidly.

We should realize that the time elapsed from the recognition of the real importance of the disease to the present is but a scant twenty-five to thirty years. Many investigators have studied brucellosis in both man and animals, but its occurrence and characteristics in children have received relatively little attention. It is not surprising that our information regarding the disease in the pediatric age group is meager, that its true incidence is unknown, and that perhaps even its clinical characterization is incomplete.

Brucellosis, presumably due to *Br. melitensis,* was recognized in chil-

dren by the early workers. Marston, in 1860, noted its occurrence. In the early bacteriologic era, practitioners in the Mediterranean area found the disease in children, although it was regarded as rare in infancy and uncommon below the age of six years.[32] With the recognition of brucellosis along the Texas-Mexican border in 1911, due to infection with the goat strain, cases falling within the older pediatric age group were reported.[20, 23] It is of interest that, prior to the knowledge of the pathogenicity of *Br. abortus* for man, Mohler and Traum,[52] in 1911, isolated *Br. abortus* from a tonsil removed from a child who had been drinking raw milk. Similarly, evidence of Brucella antibodies in children in this country was presented at an early date. In 1913 Larson and Sedgwick[40] reported complement-fixing antibodies in the blood of 17 per cent of 425 children examined. They also noted several infants with concurrent enlargement of the spleen. In summaries of all reports of brucellosis in the United States up to 1929 few instances were found in children. In Hardy's[24] studies in Iowa of a total of 354 cases investigated, there were only four in the age group 0 to four years, and a total of 27 from 0 to fourteen years of age. Hasseltine[26] reports similar data. Since 1930, both in this country and abroad, reports of brucellosis in children have continued to appear. Most of these deal with one or a few cases.

Compared to the number of adult cases reported, diagnosed cases in children have been rare, particularly below the age of six years. Even today the disease is found so seldom in young children that single case reports are published. Varying and often inadequate diagnostic criteria render evaluation of the literature difficult. However, well substantiated cases, examples of which are cited here, have been reported at every age, including the very young infant.[6, 13, 22, 30, 38, 62] More cases have been reported between the ages of two and six.[2, 13, 27, 56, 60] Reports of cases between the ages of six and fourteen years[2, 8, 12, 13, 31, 42, 55] are relatively common, and thereafter the rate begins to approach that in the adult.

By bearing in mind the difficulties of diagnosis and other factors which may limit the number of reported cases, a clearer index of the relative number of cases that may occur and greater insight into the nature of brucellosis in the child may be gained by considering serologic and epidemiologic data.

Fleischner and Meyer[21] failed to detect agglutinins in the blood of seventy-five children under five years of age drinking milk containing *Br. abortus*. Meyer,[51] studying a group of 200 children between the ages of a few weeks and five years, reported negative agglutination and cutaneous reactions in all but two. Kristensen in Denmark[39] reported the results of agglutination tests on 4623 serums representing all age groups. Five hundred yielded titers of 1:100 or higher; of these, thirty-one were from patients below the age of fifteen years (five below nine years). Bayne-Jones,[5] testing routine blood specimens, found no positive agglu-

tination reactions in 3700 serums from infants under one year. Dooley[14] reported an illuminating study of 300 institutionalized children consuming raw milk from an infected source. Few cases of low grade illness occurred, although 41 per cent developed detectable agglutinins. Interestingly enough, distinct clinical cases occurred in adults consuming the same milk. Angle et al.[3] determined the cutaneous sensitivity to Brucellergen of 7122 children in Kansas City. In the four to nine year group, of 1213 tested, 5.7 per cent were positive; in the ten to fourteen year group, 9.7 per cent of 3400 tested were positive; while in the fifteen to nineteen year group, 9.6 per cent of 2509 children were positive. At the time of testing there was a high rate of Bang's disease in the cattle of this area. These and other studies suggest that subclinical infection may be frequent in exposed children, while the overt clinical case is rare.

Magoffin et al.[50] made an extensive epidemiologic study of 268 culturally proved cases of brucellosis occurring in Minnesota from 1945 to 1948. There were only twenty cases in children twelve years of age and under. Nine of these were under ten years of age, and four under five years. This study was done in an area where brucellosis has been scientifically investigated for many years. It probably reflects fairly closely the situation obtaining in the United States. Jordan[33] presented the cases reported to the Iowa State Department of Health for the seven year period, 1940–46. There were 2405 cases of all age groups. Thirteen cases were in the one to four year age group, sixty-three in the five to nine, and fifty-five in the ten to fourteen year age group. Similar studies in The Netherlands[28] and China[64] furnish comparable data for those areas. The author's series likewise contains relatively few cases in the pediatric age group.

Brucellosis may occur in a child of any age; it is rare in infancy; and, while the incidence is far below that in the adult, the disease is not too uncommon in the older pediatric age group.

GEOGRAPHIC DISTRIBUTION

Brucellosis is worldwide in distribution in both animals and man, the disease having been reported from practically every country in the world. *Brucella melitensis* infection is largely confined to those areas of the globe where the goat industry is large, namely, the Mediterranean area, North Africa, the Near and Far East, Central and South America. It has not become widely distributed in the United States, although it has been reported in either animals or man from at least ten states. There is a low incidence of endemic infection among goats in our southwestern states bordering on Mexico, and it is present in swine of our midwestern states, particularly in Iowa and the adjacent counties of Minnesota. The distribution of *Br. abortus* follows the distribution of

cattle, particularly of dairy cattle, throughout the world. In the United States at the present time, approximately 4 per cent of dairy cattle are believed to be infected. *Brucella suis* is not so uniformly distributed. Its occurrence is best known in Central Europe and the United States and parts of South America, though it has been reported from many areas. It is variously estimated that 6 to 15 per cent of the hogs in the United States are infected.

The distribution of brucellosis and of Brucella species in man parallels the distribution in animals. In the pediatric age group there is no difference in the rates between the sexes and no racial predilection.

EPIDEMIOLOGY

Man contracts brucellosis through direct contact with infected animals or their tissues or from consuming dairy products or other animal products containing the organism. It is, then, an occupational disease affecting the worker who comes in contact with infected animals, such as the farmer, stock breeder, dairyman, butcher, abbatoir and packing house worker, and veterinarian. In recent studies in the United States[33, 50] it is estimated that approximately three fourths of our cases of brucellosis are due to such direct contact and about one fourth are milk-borne. The disease is primarily one of the third, fourth and fifth decades of life, with three fourths of the cases in males, reflecting the greater exposure of the adult male to infected animals.

While *Br. melitensis* is usually found in the goat or sheep, *Br. abortus* in the cow, and *Br. suis* in the hog, they are not strictly confined to these respective species, and any animal may be infected with any of the three. The animal exposure history, then, is not a reliable guide to the identity of a Brucella species infecting man. Most of our milk-borne epidemics of brucellosis have been due to *Br. suis*, the sporadic cases to *Br. abortus*.[33] *Brucella melitensis* infections in the mid-West derive from infected hogs,[7, 34, 35] as do some of the cases due to *Br. abortus*.[46, 47] Most infected animals shed Brucella in the milk at some time or other, and some animals continue to shed the organism over long periods of time and through many lactation periods. We must regard every infected lactating animal as a potential source of milk-borne disease. In addition to milk, butter, ice cream and cheese serve as sources of infection. Soft curd cheeses, especially those made from goat and sheep milk, are particularly dangerous. Adequate pasteurization, of course, renders milk and other dairy products safe. Aside from ingestion of the organism, the infection may enter through the mucous membrane of the mouth, nose and throat, eye, or through any cut or abrasion of the skin. There is even good evidence that the organism may pass through the unbroken skin.[53] Though airborne infections can

be easily produced in laboratory animals, there is no real evidence that air spread occurs significantly in nature. The horse and other animals may occasionally be the source of infection.

The question of intrauterine transmission of brucellosis is of possible interest. Carpenter et al.,[11] in 1927, first isolated Brucella *(Br. abortus)* from an aborted human fetus. Others have since occasionally done so. To the author's knowledge, there is no case on record of a child born of an infected mother and clearly shown to have become infected prior to birth. This is, of course, theoretically possible and probably occurs occasionally. Brucella has been isolated from the milk of lactating infected mothers,[19] and children nursing from such mothers have been found to have the disease.*

PATHOLOGY

When Brucella organisms enter the body, by whatever route, they soon reach the regional lymph nodes, and subsequently become distributed throughout the body. The incubation period varies from five days to several months and is usually between two and four weeks. The organisms prefer an intracellular environment and are found in the liver, spleen, lymph nodes and other reticuloendothelial sites. From the onset of clinical illness, organisms are present in the blood, though usually in small numbers and often only intermittently. Histologically, the tissue changes are nonspecific. Focal necrosis and cellular infiltration of affected areas are seen, as with other systemic infections. The liver almost always shows such changes. Granulomas,[1, 25, 45, 54, 57] usually microscopic in size, may be found in the liver, spleen, lymph nodes and bone marrow. They are usually scanty and must be searched for, but can nearly always be found. They are not distinguishable with certainty from those due to other granulomatous disease, such as sarcoidosis or tuberculosis. There is usually little or no necrosis, the granuloma consisting of compact epithelioid cells, surrounded and sparsely infiltrated with round cells and plasma cells. Polymorphonuclear cells may be present, though usually not in great numbers. Occasional giant cells of the Langerhans type may be seen.

Gross pathology is usually scanty. Enlargement of the spleen, usually without gross inflammatory foci, and lymphadenopathy, regional or generalized, are usually present in the acute case. In chronic disease gross pathologic changes may be lacking or confined to those cases with complications. Numerous complications are seen, though not all of them have been noted in children. Abscesses may occur in any organ. Osteomyelitis may consist of minute radiolucent areas or extensive lesions with serious destruction of bone. The lumbosacral vertebrae are common sites

* M. R. Castaneda: personal communication.

for such lesions. Destruction may be sufficient to cause collapse and gibbus formation requiring spinal fusion. There may be an accompanying psoas or other soft tissue abscess. Subperiosteal abscesses may occur, more usually on the ribs. Localization in a single joint with swelling and effusion is not rare. The organism has been recovered from localized processes in practically every organ of the body, such as ovarian cyst, gallbladder, prostate, testicle, cerebrospinal fluid and meninges and from vegetations of the heart valves. Cirrhosis of the liver may follow a Brucella hepatitis.[45] Chronic local lesions are said to occur most frequently in infections due to *Br. suis* and least with *Br. melitensis*. *Brucella abortus* is not infrequently the cause. The author has seen serious bone destruction with all three species.

CLINICAL ASPECTS

Brucellosis is a generalized infection due to one of the three species of Brucella. Though in a statistical series minor differences in the average clinical course and complications may be recognized, the disease produced is essentially the same, and the infecting species cannot be determined on clinical grounds. All the features ascribed to brucellosis may be produced by any of them.

Brucellosis is a protean disease, and the clinical course is variable. In general, the disease is milder in children than in adults. The onset may be abrupt or gradual. While reported series of cases in children are not extensive, probably the majority of cases have a fairly abrupt onset with headache, chills and fever, profuse sweating, weakness and generalized aching. The disease may last for a few days or for weeks and months. There may be one or more febrile periods with remissions, although the relapsing or undulant fever type of disease would appear to be rare in children. Usually, there is an abrupt onset with a single bout of fever declining gradually and lasting for a few days to several weeks, or there is a gradual onset of low grade irregular fever, with elevation more commonly occurring in the late afternoon or evening, persisting over a number of months. In young children and infants, fever may be the only finding. Other cases resemble an upper respiratory infection or influenza which is followed by a period of low grade irregular fever.

The throat may be moderately reddened, and a nonproductive cough may be present. Diarrhea may occur and would seem to be fairly common in children below the age of six and almost always present in the infant. Abdominal discomfort and vague abdominal pains are present in about half of the cases. Symptomatic arthralgias without objective findings are common. Sweating, particularly night sweats, is nearly always present, even in the young infant. Occasional cases in children have been described in which sweating was not noted. Anorexia is common in chil-

dren, particularly the younger ones. A mother often complains that a young child or infant has been irritable and feverish and slept poorly, has taken food only with urging, has appeared to have abdominal distention and distress, passed several loose stools and perspired profusely at night. The temperature may range as high as 104° F.

Physical examination is noteworthy by the paucity of findings. There may be no abnormal findings whatever. In about half of the cases the spleen is slightly enlarged and may be tender, and there may be generalized lymphadenopathy. To prevent confusion, it should be mentioned that spots resembling "rose" spots occurring over the abdomen and also a nondescript maculopapular rash have been described. Both are unusual. A variety of allergic skin manifestations have been noted. The history and physical findings are not sufficient to allow of clinical diagnosis, and one must rely upon laboratory tests to establish the presence of the disease.

The fatality rate is not accurately known, but is low, probably from 1 to 5 per cent in untreated cases, varying with the type of infection, age, the hygienic and nutritional state, and the presence of other disease or complications. Recovery without sequelae is the rule.

LABORATORY DIAGNOSIS[43, 44, 58] AND X-RAY FINDINGS

Culture of the Organism

Special methods and properly qualified personnel are a necessity if diagnosis by recovery of Brucella in culture is to be done successfully. The clinician should assure himself that the laboratory providing this service is dependable in this respect. With proper cultural techniques the organism can be isolated from the blood of most patients when fever is present. Daily cultures should be taken, preferably for seven to ten days. Three such cultures are suggested as the practical minimum to allow recovery of the organism from the majority of patients with illness of short duration. Sternal marrow culture frequently yields the organism when blood cultures are sterile. In long-standing disease the removal and culture of a suitably enlarged lymph node are of great value. Surgical specimens, cerebrospinal fluid and other secretions and exudates may occasionally serve as sources for culture. Recovery of Brucella from the patient, of course, constitutes the only real proof of the diagnosis.

Agglutination Test

In the absence of a positive culture, reliance must be placed on the agglutination test. This test is highly reliable if properly done. Case reports in the literature suggest that agglutinin production in children, and even in the infant, is as constantly present as in adults with brucellosis.

Diagnostic criteria for the pediatric age group are the same as those obtaining for the disease in the adult. Much confusion arose in the past and doubt has been shed on the reliability of the agglutination test, owing to the use of poor, variable and nonstandardized antigens. To overcome this difficulty and to promote more uniform and reliable diagnosis a standard antigen[59] has been designated. This antigen, prepared according to the recommendations of the National Research Council, is now available through a commercial source. It should be adopted and used by all laboratories.

Likewise, a standard method for conducting the test is important. The tube dilution method with forty-eight hour incubation at 37° C. is recognized as the standard method. Various rapid or slide agglutination techniques are available, but none of them provides results which can regularly be correlated with those obtained by the tube dilution method. They may be used for rapid screening, but reported titers should always be determined by the standard method. There is no "diagnostic titer," but titers of 1:320 or higher are found in most cases. Lower titers need not be ignored, but do not of themselves provide adequate presumptive confirmation of a clinical diagnosis. A rising titer is usually significant, and in chronic cases a fluctuating titer may be found. Cross reactions occur with the causative agents of Asiatic cholera and tularemia. Prior vaccine therapy may confuse the interpretation of the test for months thereafter, and even the stimulation resulting from antigen used in the skin test may produce agglutinins which persist for a variable time.

Cutaneous Sensitivity

Cutaneous sensitivity usually develops in brucellosis after several weeks, although a small percentage of patients fail to become sensitized. Large numbers of the general population, most of whom give no history of clinical illness, have cutaneous sensitivity to Brucella. Below one year of age the incidence is low, rising gradually with age and approaching the adult rate in the ten to fourteen year age group. Conversion from negative to positive during a clinical illness has some supportive value in diagnosis, but on the other hand could be entirely incidental. A positive skin test elicited initially during an illness yields no pertinent diagnostic information concerning the current illness. The test results should be interpreted similarly to those of a tuberculin test. It is recommended that this test not be used, since it is practically valueless as a diagnostic aid.

Complement fixation and opsonocytophagic tests, tests for "blocking antibodies," the Coombs test and many others have been devised and used. None of these provides information of proved value to the diagnostician at present.

Sedimentation Rate

The erythrocyte sedimentation rate is normal or only slightly elevated.

Hematologic Examination

The total white blood cell count falls within the normal range in a high proportion of cases. In acute cases there is usually a leukopenia with a relative or absolute lymphocytosis. With counts in the normal range there is still apt to be a lymphocytosis in excess of the recognized values for a particular age. A secondary anemia is often present.

X-Ray Findings

Chest. Hilar lymphadenopathy is often noted. A small number of cases show a minimal bronchopneumonia.

Skeletal. In cases of several months' duration a skeletal survey should be made to detect destructive lesions of bone.

COMPLICATIONS

The clinician should be alert for complications. Those most noted in children are peripheral neuritis of any nerve, more commonly the sciatic; suppurative arthritis; and osteomyelitis. Fatal meningitis has been reported in the infant. Abscesses may occur in any location. Bronchopneumonia occurs in a small percentage of patients. Endocarditis and cirrhosis of the liver have not, to the author's knowledge, been described in children. Hepatitis with enlargement of the liver is not infrequent; if it is severe, jaundice may supervene. Ocular inflammatory lesions have been attributed to Brucella infection, but the etiologic relationship is far from clear.

CLINICAL MANAGEMENT

Effective treatment for brucellosis is now available. The first such treatment, the combined use of sulfadiazine and streptomycin, has been discarded, owing to the high incidence of toxic reactions which occurred and the advent of more effective treatment.

The broad-spectrum antibiotics, used singly, promptly suppress the disease, and the patient becomes afebrile and asymptomatic within a few days. The relapse rate is high, however, in some series ranging up to 70 to 80 per cent.[37, 48] The use of these drugs singly is not recommended.

A combination of dihydrostreptomycin with chlortetracycline or oxytetracycline has proved effective.[29, 49] The relapse rate is low, probably less than 5 per cent. The author prefers the combination of dihydro-

streptomycin, chlortetracycline and sulfadiazine (or triple sulfonamide).[15-17] In the author's series of thirty-five cases treated with this combination and followed up for two to four years, there have been no relapses. Both acute and chronic cases, and all three species of Brucella, were represented in the series. This combination of antibiotics, with or without the addition of a sulfonamide, is the treatment of choice at the present time. Tetracycline has been used as the broad-spectrum antibiotic in this combination* on a few cases and results have been good, but sufficient cases have not accrued to warrant recommending it at present.

Even these combined treatments are mainly suppressive in action, and treatment must be intensive and prolonged to assure a cure.

In the adult, dihydrostreptomycin is given in a dosage of 2 gm. a day (0.5 gm. intramuscularly every six hours) and chlortetracycline 2 gm. a day in divided doses (0.5 gm. orally every six hours). If sulfadiazine is added, it is given in sufficient dosage to yield a 10 mg. per 100 ml. blood level, usually 4 to 6 gm. a day. The sulfonamide is commenced the day prior to the antibiotics, which may be given in reduced dosage on the first day of administration to avoid undue intolerance.

Dosage in children is reduced appropriately. A suggested schedule for the younger ages is dihydrostreptomycin, 40 mg. per kilogram per day; chlortetracycline, 30 mg. per kilogram per day; and sulfonamide to yield a blood level of 10 mg. per 100 ml., usually 0.15 gm. per kilogram per day. Patients weighing 100 pounds or more may receive the adult dosage. The patient should be closely observed for gastrointestinal disturbance or possible toxic effects on the eighth nerve. If severe intolerance develops, the dosage may be reduced or stopped for a few days and then resumed.

Treatment is continued for three weeks in the uncomplicated case. In a case of long standing, or if relapses have occurred or complications are present, longer treatment is necessary. Destructive bone lesions or local abscesses, of course, require surgical treatment.

Bed rest throughout treatment and general supportive measures are important and should not be neglected. Adequate diet and vitamin supplements should be given, and fluid output be maintained at a high level.

COMMENT

Many cases of brucellosis in children escape detection because the disease is not considered. Brucellosis should be included in the differential diagnosis in every case of unexplained fever persisting for more than a few days, particularly if a possible exposure history is elicited. A normal erythrocyte sedimentation rate, together with a normal or low

* N. B. McCullough: unpublished data.

white blood cell count with a relative or absolute lymphocytosis, should suggest consideration of brucellosis. Nontender lymphadenopathy and fever, or a monoarticular arthritis or cold abscess in the absence of tuberculous implications, likewise are suggestive.

REFERENCES

1. Albertini, A. von, and Lieberherr, W.: Beiträge zur pathologischen Anatomie der Febris undulans Bang. Frankfurt. Ztschr. Path., *51*:69, 1937.
2. Anderson, E. D., and Pohl, J. F.: Undulant Fever in Children (Report of Three Cases). Am. J. Dis. Child., *42*:1103, 1931.
3. Angle, F. E., Algie, W. H., Baumgartner, L., and Lundsford, W. F.: Skin Testing for Brucellosis (Undulant Fever) in School Children. Ann. Int. Med., *12*:495, 1938.
4. Bang, B.: Die Aetiologie des seuchenhaften ("infectiosen") Verwerfens. Ztschr. f. Tiermed., *1*:241, 1897.
5. Bayne-Jones, S.: Agglutination Tests for the Diagnosis of Undulant Fever. Am. J. Pub. Health, *20*:1313, 1930.
6. Black, R. A.: *Brucella Melitensis* Meningitis in an Infant. Arch. Pediat., *54*:702, 1937.
7. Borts, I. H., McNutt, S. H., and Jordan, C. F.: *Brucella Melitensis* Isolated from Swine Tissues in Iowa. J.A.M.A., *130*:966, 1946.
8. Boyd, H., and Prignano, J.: Brucellosis Treated by Aureomycin: Case Report. Connecticut M. J., *13*:435, 1949.
9. Bruce, D.: Note on the Discovery of a Microorganism in Malta Fever. Practitioner, *39*:161, 1887.
10. Bruce, D.: Sur une nouvelle forme de fièvre recontrée sur les de la Meditérrannée. Ann. Inst. Pasteur, *7*:289, 1893.
11. Carpenter, C. M., and Boak, R. A.: Isolation of *Brucella Abortus* from a Human Fetus. J.A.M.A., *96*:1212, 1931.
12. Cruikshank, J. C., and Stevenson, G. A.: Undulant Fever—a Small Outbreak in a Girls' School. Brit. M. J., *1*:522, 1942.
13. Dietrich, H., and Bonynge, C. W.: Undulant Fever in Childhood. J. Pediat., *1*: 46, 1932.
14. Dooley, P.: The Incidence of Undulant Fever in Children. Arch. Dis. Childhood, *6*:235, 1931.
15. Eisele, C. W., and McCullough, N. B.: The Treatment of Brucellosis. Illinois M. J., *96*:241, 1949.
16. Eisele, C. W., and McCullough, N. B.: Treatment of Brucellosis (Undulant Fever), in Conn, H. F.: Current Therapy 1950. Philadelphia, W. B. Saunders Company, 1950, p. 5.
17. Eisele, C. W., and McCullough, N. B.: Discussion of Herrell, W. E., and Barber, T. E.[29]
18. Evans, A. C.: Further Studies on *Bacterium Abortus* and Related Bacteria. II. A Comparison of *Bacterium Abortus* with *Bacterium Bronchisepticus* and with the Organism Which Causes Malta Fever. J. Infect. Dis., *22*:580, 1918.
19. Eyre, J. W. H.: Reports of the Commission for the Investigation of Mediterranean Fever. London, Harrison and Sons, 1907, Part VI, p. 127.
20. Ferenbaugh, T. L.: Endemic Malta Fever (Mediterranean Fever) in Southwest Texas. J.A.M.A., *57*:730, 1911.
21. Fleischner, E. C., and Meyer, K. F.: The Bearing of Cutaneous Hypersensitiveness on the Pathogenicity of the *Bacillus Abortus Bovinus*. Am. J. Dis. Child., *16*:268, 1918.
22. Fliess, M. M., and Jordan, J. V.: *Brucella Abortus* Infection in Early Infancy. J. Pediat., *3*:502, 1933.
23. Gentry, E. R., and Ferenbaugh, T. L.: Endemic Malta (Mediterranean) Fever

in Texas with the Isolation of the *Micrococcus Melitensis* from Two Patients. J.A.M.A., 57:889, 1911.
24. Hardy, A. V., Jordan, C. F., Borts, I. H., and Hardy, G. C.: Undulant Fever, with Special Reference to a Study of Brucella Infection in Iowa. Nat. Inst. Health Bull. 158, 1930.
25. Haslhofer, L.: Zur Kenntnis der Gewebsveranderungen bei der Bangschen Erkrankung des Menschen. Virchows Arch. f. path. Anat., 291:912, 1933.
26. Hasseltine, H. E.: A Study of the Epidemiology of Undulant Fever. Am. J. Pub. Health, 21:519, 1931.
27. Hauptmann, W., and Eberle, K.: *Bang*-Infektionen (Febris undulans *Bang*) bei Kleinkindern. Arch. Kinderh., 100:65, 1933.
28. Hemmes, G. D.: Brucellosis Bang among the Population of The Netherlands. Versl. Mededel. betref. Volksgezondheid, 4:261, 1950.
29. Herrell, W. E., and Barber, T. E.: A New Method for Treatment of Brucellosis. J.A.M.A., 144:519, 1950.
30. Hill, W. O., and Monger, R. H.: Undulant Fever in a Child under Seven Months of Age. J.A.M.A., 97:176, 1931.
31. Hubbard, J. P.: *Brucella Abortus* Infection in Infancy and Childhood. J. Pediat., 1:464, 1932.
32. Hughes, M. L.: Mediterranean, Malta or Undulant Fever. New York, Macmillan Company, 1897.
33. Jordan, C. F.: The Epidemiology of Brucellosis. Brucellosis Symposium. Washington, D.C., Am. A. Adv. Sci., 1950, p. 98.
34. Jordan, C. F., and Borts, I. H.: Occurrence of *Brucella Melitensis* in Iowa. J.A.M.A., 130:72, 1946.
35. Kabler, P., Bauer, H., and Nelson, C. B.: Human *Brucella Melitensis* Infections in Minnesota with Hogs as the Probable Source. J. Lab. & Clin. Med., 32: 854, 1947.
36. Keefer, C. S.: Report of a Case of Malta Fever Originating in Baltimore, Maryland. Bull. Johns Hopkins Hosp., 35:6, 1924.
37. Killough, J. H., Magill, G. B., and Smith, R. C.: Terramycin, Chloramphenicol and Aureomycin in Acute Brucellosis. J.A.M.A., 145:553, 1951.
38. Kohlbry, C. O.: A Case of Undulant Fever in an Infant. Minnesota Med., 12: 414, 1929.
39. Kristensen, M., and Holm, P.: Bakteriologische und statistische Untersuchungen über Febris undulans in Dänemark. Zentralbl. f. Bakt., 112:281, 1929.
40. Larson, W. P., and Sedgwick, J. P.: The Complement Fixation Reaction of the Blood of Children and Infants Using the *Bacillus Abortus* as Antigen. Am. J. Dis. Child., 6:326, 1913.
41. Löffler, W., and Albertini, A. von: Pathologisch-anatomische Befunde bei sogennanten Febris undulans des Menschen. Krankheitsforschung, 8:1, 1930.
42. McBryde, A., Daniel, N. C., and Poston, M. A.: Brucella Infection in Children. J. Pediat., 4:401, 1934.
43. McCullough, N. B.: Laboratory Tests in the Diagnosis of Brucellosis. Am. J. Pub. Health, 39:866, 1949.
44. McCullough, N. B.: Laboratory Tests in Brucellosis. Brucellosis Symposium. Washington, D.C., Am. A. Adv. Sci., 1950. p. 116.
45. McCullough, N. B., and Eisele, C. W.: Brucella Hepatitis Leading to Cirrhosis of the Liver. Arch. Int. Med., 88:793, 1951.
46. McCullough, N. B., Eisele, C. W., and Pavelchek, E.: Isolation of *Brucella Abortus* from Hogs. Pub. Health Rep., 64:537, 1949.
47. McCullough, N. B., Eisele, C. W., and Pavelchek, E.: A Survey of Brucellosis in Slaughtered Hogs. Pub. Health Rep., 66:205, 1951.
48. Magill, G. B., and Killough, J. H.: Therapy of Brucellosis in Egypt. J. Egyptian M. A., 36:447, 1953.
49. Magill, G. B., and Killough, J. H.: Oxytetracycline-Streptomycin Therapy in Brucellosis Due to *Br. Melitensis*. Arch. Int. Med., 91:204, 1953.

50. Magoffin, R. L., Kabler, P., Spink, W. W., and Fleming, D.: An Epidemiologic Study of Brucellosis in Minnesota. Pub. Health Rep., *64*:1021, 1949.
51. Meyer, K. F.: Public Health Control of Infectious Abortion in Certified Milk. Am. J. Pub. Health, *21*:503, 1931.
52. Mohler, J. R., and Traum, J.: Infectious Abortion of Cattle. Washington, D.C., Bureau of Animal Husbandry, U.S. Department of Agriculture, Circular 216, 1911, p. 157.
53. Morales-Otero, P.: Experimental Infection of *Brucella Abortus* in Man. Puerto Rico J. Pub. Health & Trop. Med., 5:144, 1929.
54. Pedro Pons, A., Bacardi Noguera, R., and Alvarez Zamora, R.: Hígado melitócocico: Estudio histopatológico del mismo; Mediante la punción biopsica aspiradora. Med. clín., *5*:15, 1945.
55. Poston, M. A., and Thomason, R. H.: Meningitis Due to *Brucella* in a Child. Am. J. Dis. Child., *52*:904, 1936.
56. Spink, W. W., Hall, W. H., and Magoffin, R.: Follow-up Study of Therapy in Forty-Eight Culturally Proved Cases of Brucellosis. Arch. Int. Med., *88*:419, 1951.
57. Spink, W. W., Hoffbauer, F. W., Walker, W. W., and Green, R. A.: Histopathology of the Liver in Human Brucellosis. J. Lab. & Clin. Med., *34*:40, 1949.
58. Spink, W. W., McCullough, N. B., Hutchings, L. M., and Mingle, C. K.: Diagnostic Criteria for Human Brucellosis. J.A.M.A., *149*:805, 1952.
59. Spink, W. W., McCullough, N. B., Hutchings, L. M., and Mingle, C. K.: A Standardized Antigen and Agglutination Technic for Human Brucellosis. Am. J. Clin. Path., *24*:496, 1954.
60. Sprunt, D. H., and McBryde, A.: Morbid Anatomic Changes in Cases of Brucella Infection in Man. Arch. Path., *21*:217, 1936.
61. Traum, J.: Infectious Abortion Investigations in Pigs. Washington, D.C., Annual Report of the Chief, Bureau of Animal Industry, U.S. Department of Agriculture, 1914, p. 86.
62. Williamson, B., and Gibson, A. J.: Undulant Fever in Infancy. Brit. M. J., *1*:748, 1931.
63. Zammit, T.: A Preliminary Note on the Examination of the Blood of Goats Suffering from Mediterranean Fever. Reports of the Commission for the Investigation of Mediterranean Fever. London, Harrison and Sons, 1905, Part III, p. 83.
64. Zia, S. H., and Wang, F.: Brucellosis in North China. A Clinical, Etiological and Epidemiological Study. Am. J. Trop. Med., 29:925, 1949.

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LEPTOSPIROSIS

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The first vivid description of leptospirosis was published by Weil, a German physician, in 1886,[16] but at that time the disease could not be distinguished from other forms of severe febrile illness with muscle aching, jaundice and hemorrhagic manifestions. It was not until 1915 that Inada demonstrated the causal agent to be a spirochete.[4] In the next three years Noguchi separated this spirochete from other forms and placed it in a new group which he renamed *Leptospora icterohaemorrhagiae*.[11, 12]

For almost fifty years after Weil's original report the disorder was clinically recognized throughout Europe and Asia, but no serviceable methods for establishing an exact etiologic diagnosis were available. It was called by a variety of names, most of which included the feature symptom of jaundice. It gradually became evident that it was primarily an epizootic disease of rodents, dogs and other animals. The common sewer rat, *Rattus norvegieas*, was recognized as the chief host. Human infection was known to occur through contamination of food and water by leptospira shed in rat urine.

The first instance of leptospira infection in a human case in the United States is Stimson's description of autospy material from a jaundiced patient in New Orleans believed to have died of yellow fever. Thirty-three years later Sellard[13] re-examined the tissues of this patient and demonstrated beyond a doubt that the "spirochetes" reported by Stimson were in fact leptospira. He published an illustration of the kidney which clearly shows the typical organisms. A large group of cases of Weil's disease was reported in 1941 by Larson,[6] and many others have been published since that time.

During the past decade serum agglutination and complement fixation studies have largely replaced the demonstration of leptospira in blood, urine and cerebrospinal fluid, and positive animal inoculation experiments in the clinical diagnosis of Weil's disease and similar types of leptospira infections. These serum tests have afforded clinicians the

means of readily establishing a positive etiologic diagnosis and thereby have increased tremendously the number of cases reported yearly. As leptospirosis has an almost universal rodent distribution, cases in man have been reported from almost every country in the world, including many infections in the United States. Commercially prepared antigens suitable for serum agglutination tests are now available, so that no medical center need be without this aid in the diagnosis of "fever of unknown origin" or "infectious jaundice."

With the perfection of serodiagnosis some fifteen widely recognized different serotypes have been identified in addition to the *Leptospira icterohaemorrhagiae* of Weil's disease. Chief of these is the *L. canicola*, which is commonly encountered in dogs throughout the world. Man's close association with the dog has produced a number of human infections with this type of leptospira, the first cases having been described in Holland in 1933,[5] and the first cases in the United States in San Francisco in the following year by Meyer et al.[8, 9] Additional types of leptospira have been identified not only in a variety of rodents, but also in horses, cattle and swine, and recently some of these types have also been shown to be the causative agent of heretofore undiagnosed fevers in man. The history of leptospirosis during the course of a few decades of time reveals that infections in the rat which spread to man in the form of Weil's disease can now be extended to infections in a wide range of animal hosts by many different serotypes of leptospira which cause a variety of febrile illnesses in the human race.

The recent history of leptospira infections can best be brought up to date by calling attention to a few publications which have appeared in the last two years and which cover the whole subject most admirably. Chief of these is the "Medical Science Publication No. 1,"[15] which contains a symposium on the leptospiroses which should serve as a standard reference for everyone interested in this subject. *The Laboratory Diagnosis of Leptospirosis*, a small book by Wolf,[17] has just appeared. Two references to antibiotic therapy should be noted,[3, 10] so that the clinician can better evaluate this important phase of the subject. Good standard texts are those of Shattuck[14] and Manson-Bahr.[7]

GEOGRAPHIC DISTRIBUTION AND EPIDEMIOLOGY

Medical literature reports are now appearing on Weil's disease, canicola fever and other serotypes of leptospira infection from nearly every country in the world. *Leptospira icterohaemorrhagiae* is encountered almost universally; however, it has not been found in examination of the rat population of Egypt and of South Africa. Additional forms of leptospirosis are less cosmopolitan and may be sharply localized geographi-

cally. In the United States *L. icterohaemorrhagiae* has been identified in the common carrier the rat, in as high an incidence as 45 per cent of 1643 rats examined. Dogs and mice also harbor this organism. There is a similarly high carrier incidence of *L. canicola* infection reported in dogs, based on high antibody titers in 15 to 40 per cent of dog populations analysed. An additional half dozen serotypes of leptospira have been incriminated as the cause of illness in this country in animal and man.

There are three chief sources of contagion for man. One is infected excreta from rodents, particularly rats and mice, urine of older dogs, and more rarely animals such as swine, horses and cattle which pollute water, kitchenware or food. Another is intimate animal contact, particularly a sick dog; rarely through animal bite. The third course of contagion, which may be an occupational risk, is contact with damp, muddy soil, or stagnant water such as occurs in rice paddies, or an infected stream or lake through swimming. Bathing in contaminated water has caused a number of group infections. Hazardous occupations are those of sewer workers, warehouse employes, slaughterhouse attendants, dock workers, miners, ditch and canal diggers, cane and rice field workers, veterinarians, dog breeders, pet shop owners, and owning "man's friend," the dog.

The only known route of human infection is through maceration, abrasions, scratches or cuts in the skin or mucous membranes. Ingestion of the organisms might conceivably produce an infection. Infection by inhalation is not known to occur. Leptospirosis readily spreads from animal to animal, presumably through close contact, so that a vast reservoir of infected hosts is maintained. Animals may die of the disease, have a severe, mild or inapparent infection, or act as carriers. As far as is known, insects play no part in the spread of this disease.

Occupation and animal friendship are more the determining factors of infection than any predilection of the disease for race, sex or age. It has been noted in Switzerland, however, that leptospirosis is largely a disease of young adult males and is more common in the hot summer months.[15] Relative to childhood infection, 2.6 per cent of 657 cases occurred under ten years of age and an additional 25.8 per cent occurred before the twentieth year.

PATHOLOGY

The pathologic findings of leptospiral infection in man vary only slightly with the nature of the disease. Practically all autopsy reports are based on cases of Weil's disease. Grossly, the characteristics are jaundice, hemorrhage and enlarged kidneys, with frequent enlargement of the liver and spleen. Bleeding in the form of petechiae and ecchymoses of the serous membranes and less often in the mucous membranes is a

consistent finding. Usually the kidneys are markedly enlarged with a bile-stained swollen cortex. The liver is only slightly increased in size as a rule, and the spleen is not uncommonly of normal size.

Microscopically, there is an interstitial nephritis; some degree of non-specific hepatic cell changes in the liver, except for the not infrequent presence of characteristic "giant nuclei"; cell structure of an acute splenic tumor and more rarely a slight degree of myocarditis. Examination of the brain may reveal a perivascular inflammation of the smaller vessels, in scattered areas. Bronchopneumonia is a frequent complication.

Of utmost importance in the pathologic examination is the application of the silver impregnation method (Levaditi stain) for the demonstration of leptospira, particularly to tissue from the kidney, liver and spleen.

CLINICAL PICTURE

Most types of leptospirosis infection which occur in the United States present similar clinical findings which vary from those found in the severe and fatally jaundiced patient with Weil's disease which is caused by *L. icterohaemorrhagiae*, to those of the patients with a mild dengue-like fever known as swineherd's disease, caused by *L. pomona*. The features of this group of disorders are fever; the variability in the intensity of symptoms; the constancy of head, back and leg aching; the frequent presence of postorbital ache, conjunctivitis and photophobia; the danger of death in jaundiced cases and the relative benign nature of nonjaundice infections.

The onset of the illness is usually abrupt, so much so that a patient will often name the exact hour his wretchedness started. It follows a completely symptomless incubation period of about eight days which may vary between extremes of two and twenty days. There is a rigor with a rapid rise in temperature to 102°, 103° or even 105° F. Chills frequently develop and can be as severe as those encountered in malaria, as can the profuse sweating which follows. Headache, backache and leg aching may be unbearable and out of all proportion to that seen in many other forms of acute infection. Arm aching occurs less frequently. Many patients have pain and stiffness of the back of the neck, aching of the eyeballs with pain on movement and photophobia, and a few have isolated muscle and joint pains, particularly at the onset. Groups of muscles are sensitive to pressure. Gastrointestinal symptoms such as loss of appetite, nausea, vomiting, constipation or diarrhea occur to a slight degree as a rule, but are pronounced in some instances. Epistaxis and cough are not uncommon. Hemoptysis is a rare manifestation. Mild degrees of mental confusion may accompany the headache.

During the first days of the infection physical examination often re-

veals only a prostrated, more or less weak and toxic patient with chills, fever and sweating and few other signs of illness. However, certain significant signs may be present as early as the first day. These are effusion of the conjunctivas which may be striking; marked tenderness of the eyeballs on pressure; pharyngeal erythema without pronounced soreness of the throat; definite stiffness of the neck; and slight to extreme muscle tenderness to pressure which characteristically involves the calf muscles. Pronounced conjunctivitis with acute myositis may be considered cardinal signs of leptospirosis at the onset. Abnormal lung signs, lymph gland adenopathy, enlargement of the liver and the spleen and altered nerve reflexes are not ordinarily found at this stage of the disease.

The febrile state may continue for a few days only or for two to three weeks, but characteristically in Weil's disease it lasts about one week. Toward the end of this week or in the second week of illness additional symptoms and signs may make their appearance. Jaundice develops in about one third of the cases, and the liver may become palpable and slightly tender. Bleeding into the skin and mucous membranes in the form of petechiae and ecchymoses occurs and is aptly described as a hemorrhagic rash. Hematemesis and melena occasionally develop. During the second week evidence of renal involvement is frequent, and as a result albuminuria, oliguria and even anuria occur. Hypotension, signs of circulatory failure, and uremia accompany advanced kidney damage. Patients with extensive hepatorenal disease tend to become comatose and die. Lung rales indicative of a bronchopneumonia develop not infrequently and may be transitory. A late pneumonia is occasionally fatal. The appearance of an encephalomeningitis offers a poor prognosis, but not necessarily a hopeless one.

This same train of clinical events can appear in other forms of leptospirosis in the United States, but is far less likely to do so. Jaundice has rarely been recorded in human infections with *L. canicola*, *L. pomona* and *L. autumnalis* in this country. These leptospiral infections in man are not fatal unless complications such as bronchopneumonia occur. They may present the clinical picture of sepsis, the typhoid state, aseptic meningitis, influenza or grippe, and pneumonitis of the virus type. Four cases of canicola fever observed by the writer in San Francisco presented the following diverse clinical manifestations: meningitis, pneumonitis, subacute nephritis and postfebrile asthenia.

THE PROBLEM OF DIAGNOSIS

When a patient suffering from leptospirosis is initially seen during the first week of illness, the problem of diagnosis is the problem of detecting the etiology of fever of unknown origin.[2] If skin rash, pneumonitis, hepatitis or meningitis is present at this time, the range of diagnostic

possibilities is somewhat narrowed. This problem is always a fascinating one. At times its solution is most difficult. All too frequently it remains unsolved largely because specific serologic tests were not carried out during the period of convalescence or more rarely because no solution is obtainable by present diagnostic means.

Actually, an accurate diagnosis of leptospira infection can nearly always be obtained by the second week of illness, provided three principal considerations are kept in mind. The *first* is an awareness of the existence of the disease, which is widespread georgaphically and protean in its manifestations. The *second* is a careful and painstaking history of a patient's contact with those host animals which harbor leptospira and of an occupation which might bring him into relationship with infected rat, dog or swine urine or with water or damp soil which might harbor leptospira organisms.

If either of these two elements of history even slightly suggests the possibility of leptospirosis as the cause of the patient's fever or symptoms, the *third* and most important diagnostic procedure should be carried out. Blood samples should be analysed for agglutination, lysis and complement fixation tests for leptospirosis. It must be always kept in mind that these tests are negative in the first five to seven days of illness, but are almost constantly significantly positive by the eighth to tenth day of infection. If a low titer of agglutination which cannot be considered diagnostic of leptospirosis is encountered in the second week, the test should always be repeated in five to seven days. These blood tests are usually strongly positive by the third or fourth week. In any long-standing case of fever of unknown origin, no matter what the history, these tests should be performed.

CLINICAL LABORATORY STUDIES

The clinical laboratory tests which are usually done are of little value diagnostically except in a negative way in the early stages of leptospira infections. Almost constantly in Weil's disease and in the more serious cases of the other forms of leptospira infection marked degrees of albuminuria are present. The urine sediment commonly shows epithelial cells, white blood cells, and hyaline and granular casts, and during the advanced stage of critical illness, when the amount of urine is scanty, it will also show red blood cells and cellular casts. In the presence of hepatitis a positive urine reaction for bilirubin occurs which precedes the appearance of clinical jaundice.

At first the blood cell count is normal, but a slightly hypochromic anemia develops in the prolonged and severe cases. Pronounced polymorphonuclear leukocytosis is the rule in the early days of the illness in cases of Weil's disease and may persist throughout the course of the

disease. The total white blood cell count frequently remains normal or may be slightly diminished in the less severe cases of leptospira infection. By the end of the first week there may be a relative leukopenia. Abnormal platelet counts have not been noted. Prolonged bleeding time and prolonged prothrombin and blood coagulation times may be noted in the second week of illness if there is extensive liver necrosis. An increase in the icteric index and in the serum bilirubin content of the blood develops in most instances of hepatitis. These changes in the blood may be detected before the patient is jaundiced and can serve as an early indication of impending liver damage. They may increase to high values as the intensity of jaundice advances, particularly in fatal cases. Blood creatine and urea values are increased in those patients with progressive renal damage, and in cases with complete urinary suppression they reach the high figures commonly encountered in the uremic state which may be terminal.

While many patients show signs of meningitis, records of complete data on the cerebrospinal fluid findings are rather meager.[1] Increase in fluid pressure and a moderate pleocytosis of mononuclear cells are common. The total cell count rarely exceeds 300. The protein is usually slightly elevated.

Diagnostic x-ray studies are of little value in the acute illness of leptospira infections, except for examination of the chest. After the first days of illness the chest plate may demonstrate a pneumonitis, particularly in *L. canicola* fever, and in advanced stages of all types of severe infections a bronchopneumonia can be demonstrated which may prove fatal. Hepatomegaly and splenomegaly are rarely sufficiently marked that they can be demonstrated by roentgenogram. There are no satisfactory skin tests for the early detection of leptospiral diseases.

Three types of diagnostic procedure are of inestimable value in the accurate identification of leptospirosis. The first is the demonstration of the presence of leptospira in the urine, blood or cerebrospinal fluid primarily by direct visualization and by culture. The second is by animal inoculation studies or, in fatal cases, by autopsy material examination. The third is by the development of positive serologic tests for leptospira.

The first type of procedure may produce an exact etiologic diagnosis early in the course of the illness, but has the drawbacks of requiring skilled technical aid, difficulties in interpretation, and risk of infection to laboratory workers. It is not a practical clinical procedure.

The second requires animal inoculation studies and is available for clinical use only at laboratory and research centers. Demonstration of leptospira in postmortem tissue is not always possible and comes too late for any practical value except that of proper identification of disease.

The third method, serologic diagnosis, is the one of choice and is generally available. Agglutination, lysis and complement fixation tests

can be carried out in some state Department of Health laboratories, and at the United States Public Health Laboratory Center in Bethesda, Maryland. Test tubes containing about 10 ml. of whole blood carefully packed should be promptly submitted to Bethesda by air mail completely labeled and preferably with an accompanying letter explaining the nature of the case and the reason for the test.

A positive laboratory diagnosis is based on the demonstration of a rising blood serum titer for leptospira antigen or on the demonstration of a high titer during the patient's convalescence. The titer does not become positive until the end of the first week of illness, certainly not before the sixth day. It should be clearly positive in the second week of illness, although a rise in titer may be deferred or depressed by antibiotic therapy administered before securing the blood specimen. An attempt should always be made to obtain a blood sample early in the illness which would ordinarily be proved negative so that it can be compared with the serologic findings which develop later on in the disease. A titer of 1:1000 may be considered positive, particularly if it follows a negative titer at an interval of a week or more. Positive titers usually rise to 1:10,000 or even up to 1:100,000 after recovery, and a positive titer may persist for many months or even years.

DIFFERENTIAL DIAGNOSIS

In considering the differential diagnosis of the acute disease one must include most acute febrile illnesses without the early characteristic objective findings which are found, for example, in acute streptococcal tonsillitis, the exanthemata, pneumonia or pyelitis. As a rule fever and aching are the only constant early signs of leptospirosis. This is also true of a number of virus, rickettsia and Coxiella infections. The eyeache, intense conjunctivitis and photophobia which are frequently present in leptospira infections may also be present in influenza, rubella, dengue and dengue-like fevers, infectious mononucleosis, Q fever and pappataci fever. Ordinarily the final diagnosis will have to wait the natural clinical evolution of the disease present or the identification of the causative organism by cultural methods, serum agglutination or complement fixation tests. Early diagnosis may be established in cases of infectious mononucleosis by recognition of characteristic mononuclear cells in the blood film and later by the presence of a positive heterophile antibody reaction.

Once jaundice develops, the question of infectious hepatitis or serum hepatitis will arise as well as hepatitis occurring as a complication of the other infectious diseases such as yellow fever, mononucleosis, brucellosis and Q fever. Again diagnosis may have to wait upon the evolution of the disease or upon the development of specific laboratory tests.

Signs of pneumonitis, clinical or radiologic; physical and cerebrospinal

fluid evidence of meningitis; and urinary and blood findings of renal failure will introduce consideration of other types of disease which will have to be differentiated from leptospirosis in the light of concomitant clinical signs and laboratory findings.

CLINICAL COURSE

Before the clinical types of leptospirosis had been identified serologically and named as serotypes they were often designated as three-day, five-day or seven-day fever. This descriptive nomenclature of the past tells us that most forms of leptospirosis run a relatively brief course and that they tend to recover rapidly. This is true today except for Weil's disease, Indonesian *L. bataviae* infections and some infections with *L. canicola*.

In Weil's disease the most frequently described course is eight days of fever with rapid defervescence and eight days with little or no fever, but with jaundice, hemorrhage, skin rash, signs of renal failure, and then death with uremia and cholemia, or ultimately complete recovery without residual damage to the liver or the kidney. Many variations in this "textbook" course occur. Jaundice is commonly observed in about 30 to 50 per cent of any series of cases, and while a relatively high mortality (30 per cent) may develop among the jaundiced cases, some patients die without jaundice, either in the first or second week or rarely later, with hemorrhage, circulatory failure, renal failure with uremia or a terminal bronchopneumonia.

Cases of *L. canicola* fever, which are now being reported in increasing numbers in the United States, may run a prolonged course for three or four weeks with a fever which may be septic in type; recurrent or relapsing bouts of pyrexia are not rare. The temperature chart may be distinctly biphasic and similar to that found in dengue fever. Four patients observed by the author in San Francisco suffered from fever for five, eleven, seventeen and eighteen days respectively. In the United States, patients with *L. canicola* infections have not had jaundice or uremia, and fatalities have not developed except in the presence of rare complications. *Leptospira pomona* infection (swineherd's disease) and *L. autumnalis* (pretibial dengue-like fever) run a mild, uncomplicated course as a rule. The nomenclature, animal host and the expected typical course of the disease for human leptospira infections in the United States are shown in Table 6.

Uncommon complications of leptospirosis are iritis, iridocyclitis, uveitis, phlebitis, acute pancreatitis, cerebrovascular accidents and late bronchopneumonia.

TREATMENT

Treatment measures to combat human disease due to leptospira infection can be readily divided into four catagories. The first and most

TABLE 6. *The Form and Course of Human Leptospirosis in the United States*

DISEASE	STRAIN	HOST	COMMON COURSE
Weil's disease	L. icterohaemorrhagiae	Rat, dog	Severe, often with jaundice
Canicola fever	L. canicola	Dog	Mild to severe; fever may be prolonged
Swineherds' disease	L. pomona	Swine, horse	Mild "fever of unknown origin"
Indonesian leptospirosis	L. bataviae	Rat, mouse	Severe (in Indonesia)
Pretibial dengue-like fever	L. autumnalis	(?)	Mild with leg rash; only Texas (?)

desirable form of therapy is a specific one which will terminate the infection. The second is successful symptomatic treatment, which all too often seems to be a forgotten clinical art. The third is the proper management of serious complications. The fourth is prophylactic therapy.

Specific Therapy

At present there is no known form of specific therapy which will either materially alter the course of the disease or lower the incidence of common complications or fatalities. Serum therapy and bismuth injections tried in the past are unavailing. After World War II it was thought that large doses of penicillin were beneficial if given early in the course of the infection. This supposition was based on its suppressive actions in animal infections. Each new antibiotic has in turn been subjected to therapeutic trial, and though early reports of the usefulness of individual preparations have seemed encouraging, not a single one can be selected as unquestionably effective. A report in 1951 from Puerto Rico[3] stated that treatment of sixty-seven cases of leptospirosis with antibiotics was unsuccessful, whether the patients received therapy early in the prejaundice stage or later after icterus had developed. Groups of cases in this series were treated with the following antibiotics: chloramaphenicol, Aureomycin, penicillin, streptomycin, Terramycin, Aureomycin and streptomycin, and Aureomycin and cortisone. Whether or not some of the most recently developed antibiotic preparations may ultimately prove satisfactory remains to be seen. In any given case erythromycin or tetramycin may well be given a therapeutic trial, even if only to prevent other forms of bacterial infection which might complicate the clinical course of the disease.

Symptomatic Treatment

As the main complaint is severe head, back and muscle aching, adequate analgesic therapy is indicated. In mild cases 5 or 10 grains of aspirin every four hours will suffice, but in severe cases codeine, ¼ to ½ grain, should be added to the aspirin. Larger doses of codeine may be given hypodermically every four to six hours if pain is unrelieved or vomiting is present. The dose should be reduced in treating young children. The constipating effect of codeine should not be forgotten. Demerol in doses of 25, 50 or 100 mg. given orally or hypodermically may be substituted for codeine if it is not effective or well tolerated. Only rarely should the use of opium or one of its derivatives be considered. No data are available on the use of butazolidine, cortisone or ACTH for the intractable painful ache of leptospirosis. They might well be tried. Free hemorrhage would contraindicate their use. Aching of the eyeballs,

which is a common complaint, may be lessened by the application of ice-cold compresses. In patients with severe headache and nuchal rigidity lumbar puncture with withdrawal of cerebrospinal fluid is indicated if the fluid pressure is increased. The pressure, if clearly increased, should be reduced to a low normal level (70 to 150 mm. of water). Benefit may be only temporary, so that the procedure may have to be repeated one or more times.

The patient's comfort is promoted by bed rest throughout the febrile period, by tepid sponges for high fever (103° F. or higher), and dry clothing following marked perspiration. Sedation with phenobarbitol in doses of ¼ or ½ grain every four to six hours is indicated to combat marked restlessness. Smaller doses are indicated for children.

Diet is relatively unimportant in short mild infections, but severely ill patients should be fed small amounts of soft nutritious food six times daily. Marked anorexia, nausea and vomiting may restrict food intake temporarily. If nourishment is frequently refused or rejected, 50 gm. of glucose given intravenously in 1000 cc. of water or normal saline solution once or twice daily can be used as a dietary supplement. Inadequate intake of fluid by mouth or excessive fluid loss by sweating, vomiting or diarrhea can also be combated by intravenous injection. If the urine output is over 1000 cc. daily, the patient's fluid intake is probably adequate.

Patients who are critically ill with leptospirosis are prostrate, toxic, occasionally slightly delirious, and exceedingly uncomfortable, particularly when chills, high fever, aching and sweats are present. They frequently present a difficult feeding problem and require repeated injections of intravenous solutions, and also transfusions if bleeding is extensive. Coma may develop. Consequently good nursing care is of paramount importance in this group of patients. It should never be neglected.

Treatment of Complications

Complicating features of leptospirosis require additional care. The detection of jaundice indicates a hepatitis which may prove fatal or may last four to six weeks with recovery. The sooner the jaundiced patient receives a high intake of carbohydrates and vitamin B complex in full therapeutic doses by injection, the better. After the acute illness is over an amino acid such as choline in doses of 0.5 gm. three times a day may be a helpful adjunct in the restoration of normal liver function. With extensive liver necrosis, hypoprothrombinemia tends to develop which can precipitate dangerous bleeding. To combat this vitamin K should be given in daily doses of 5 mg. If hemorrhage occurs and the laboratory reports a greatly prolonged prothrombin time, the intravenous injection of 50 mg. of vitamin K three or four times daily may control free bleeding.

Extensive bleeding from the mucous membranes or into the skin which occurs in the first week or ten days of illness is more likely due to a toxic effect of the infection on the blood vessels than to hepatic disease and requires treatment by the free use of blood transfusions. Blood loss due to extensive liver disease resulting in uncontrollable vitamin K deficiency will also require blood transfusions.

Extensive renal involvement which leads to suppression of urine and at times to uremic coma and death is due to leptospira involvement of the kidney tubules and requires careful attention to fluid electrolyte balance. The usual measures to combat acute nephritis are indicated, but are likely to prove fruitless.

Prophylactic Therapy

Prophylactic therapy consists in avoiding those occupations and associations which may bring one into contact with infected animals or polluted water, food or soil; care in handling sick animals which may harbor the leptospira; and the consideration of preventive vaccination for persons who may run the hazards of occupational exposure. Miners in Japan have been successfully vaccinated, so that the incidence of leptospira infection has been significantly reduced.

SUMMARY

No concise summary or list of conclusions can indelibly etch on the physician's mind the complete clinical picture of leptospirosis. Only a thoughtful perusal of the epidemiology, symptoms, signs and characteristic laboratory findings herein recorded will enable the practitioner of medicine to be ever on the alert to the presence of this widespread form of infection.

REFERENCES

1. Beeson, P. B., and Hankey, D. D.: Leptospiral Meningitis. Arch. Int. Med., 89:575, 1952.
2. Cheney, G., and Azanaz, G.: The Identification of Tropical Fevers of Virus and Rickettsial Origin. Stanford M. Bull., 4:104, 1946.
3. Hall, H. E., and others: Evaluation of Antibiotic Therapy in Human Leptospirosis. Ann. Int. Med., 35:981, 1951.
4. Inada, R., and Ido, Y.: Tokyo Ijinshinshi No. 1908, 1915.
5. Klarenbeek, A., and Schuffner, W.: Appearance in Holland of Leptospira Differing from Weil Strain. Nederl. tijdschr. v. geneesk., 77:4271, 1933.
6. Larsen, C. L.: Weil's Disease. A Report of 51 Cases Occurring in Puerto Rico and the United States. Pub. Health Rep., 56:1650, 1941.
7. Manson-Bahr, P. H.: Manson's Tropical Diseases. 13th ed. Baltimore, Williams & Wilkins Company, 1950.
8. Meyer, K. F., Eddie, B., and Stewart-Anderson, B.: Canine, Murine, and Human Leptospirosis in California. Proc. Soc. Exper. Biol. & Med., 38:17, 1938.

9. Meyer, K. F., Stewart-Anderson, B., and Eddie, B.: "Canicola Fever," a Professional Hazard. J. Am. Vet. M. A., 46:332, 1938.
10. Minor, R. W., ed.: Use of Antibiotics in Tropical Diseases. Ann. New York Acad. Sc., 55:967, 1952.
11. Noguchi, H.: *Spirochaeta Icterohaemorrhagiae* in American Wild Rats and Its Relation to the Japanese and European Strains. J. Exper. Med., 25:755, 1917.
12. Noguchi, H.: Etiology of Yellow Fever. II. Transmission Experiments on Yellow Fever. J. Exper. Med., 29:565, 1919.
13. Sellard, A. W.: The Interpretation of (? Spirochaeta) Interrigans of Stimson (1907) in the Light of Subsequent Developments. Tr. Roy. Soc. Trop. Med. & Hyg., 33:545, 1940.
14. Shattuck, G. C.: Diseases of the Tropics. New York, Appleton-Century-Crofts, Inc., 1951.
15. Symposium on the Leptospiroses. Medical Science Publication No. 1. Washington, D.C., U.S. Government Printing Office, 1952.
16. Weil, A.: Ueber eine eigenthümliche mit Milztumor, Icterus und Nephritis einhergehende acute Infectionskrankheit. Deutsche Arch. f. klin. Med., 39:209, 1886.
17. Wolf, J. W.: Laboratory Diagnosis of Leptospirosis. To be published.

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RAT-BITE FEVERS

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Rat-bite fever is an acute, febrile disease of man occurring most often in infants and children as a result of wound infection from the bite of a rat. It is characterized by a local lesion at the site of the bite with regional lymphadenopathy, intermittent fever, chills and a skin rash. The disease is widespread and naturally prevails in those countries where the rodent population is greatest and modern sanitation is not practiced.

The first accurate description of rat-bite fever is credited to Wilcox,[25] who in 1840 reported the first case of the disease in the United States. In 1900 Miyake[17] reported the disease in Japan as an entity long known in that country and designated as Sodoku (*So*, a rat, and *doku*, poison). Excellent reviews of rat-bite fevers in the United States have been published by Bayne-Jones,[3] Brown and Nunemaker,[6] Beeson,[4] Watkins[22] and Griffith and McNaughton.[12] An ever-increasing number of cases are being reported in the medical literature, owing to better recognition of the disease.

ETIOLOGY

Two entirely different infectious agents are now recognized as causes of the syndrome: (1) *Streptobacillus moniliformis* and (2) *Spirillum minus*. In the United States each agent has caused approximately the same number of reported cases. Both organisms have been recovered from a few cases. A few investigators hold to the opinion that the former is the specific cause of the disease and that *Spirillum minus* is only a commensal factor in the host-parasite relationship. On the other hand, several observers believe that *Spirillum minus* is the true etiologic agent

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and that *Streptobacillus moniliformis* is a secondary infection. That two infectious agents, so different biologically, can cause similar entities is a striking paradox. *Streptobacillus moniliformis* is present in the mouth of normal wild rats, and *Spirillum minus* can be readily demonstrated in the blood of many species of wild rats. Thus rats serve as the chief reservoir of infection. *Spirillum minus* has also been detected in the blood of mice, moles and bandicoots.

Streptobacillus moniliformis was first so named by Levaditi, Nicolau and Poincloux[15] in 1925, although it has been described under several names. The organism is a microaerophilic, gram-negative, pleomorphic, nonmotile, nonspore-bearing rod varying from 2 to 3 microns in length and from 0.2 to 0.5 micron in width. The morphology varies from a coccobacillus to long slender rods which may show branching and often beading. Primary cultures may be isolated on a medium enriched with blood, serum or ascitic fluid and incubated at 36° to 36.5° C. in an atmosphere containing 10 per cent carbon dioxide. Experimental infections may be established in mice and in embryonated eggs, localizing particularly in the joints of the chick embryo.

Spirillum minus, a spirochete-like organism, was first reported as the cause of rat-bite fever in 1917 by Futaki, Takaki, Taniguchi and Osumi[10] and named by them *Spirochaeta morsus muris*. Because it had been previously observed in the blood of a rat in India by Carter in 1877 and designated as *Spirillum minus*, this is now recognized as the correct name. The organism is comprised of coils, the number depending upon its length, which varies from 2 to 5 microns. The width of the coil is approximately 0.2 micron. Opinions differ concerning whether a single flagellum or a tuft of them is at each end. *Spirillum minus* is readily observed microscopically by darkfield illumination. A rapid, darting to-and-fro motion characterizes their motility. Few investigators have claimed the successful cultivation of *Spirillum minus*, but most microbiologists doubt whether the organism has ever been cultivated in vitro. It is gram-negative and may be satisfactorily stained with Giemsa's and Wright's techniques and by the standard methods of silver impregnation. Guinea pigs and mice are susceptible to the infection.

INCIDENCE

Cases of rat-bite fevers have been reported from nearly every country in the world and from most of the states of the United States. Cases due to *Streptobacillus moniliformis* are rare west of the Mississippi River; however, Haverhill, Massachusetts, experienced an epidemic in 1926 which was due to food contaminated with *Streptobacillus moniliformis*, but no other similar epidemic has been reported since.

The incidence of the two main forms of this disease appears to be

higher in infants and children than in adults. Richter[18] reported ninety-three persons in an area of Baltimore bitten by rats in a four year period. Sixty-five of the patients were observed in hospital, and only seven, or 7.5 per cent, had rat-bite fever. The majority were babies under one year, and most of the bites were on the face and hands. A similar experience was reported by Watkins[22] from St. Louis, where the incidence of reported bites is about fifty yearly, and but sixteen cases were diagnosed in an eight year period—an incidence of about 3 per cent.

The incidence of the spirillum and streptobacillus in rats varies a great deal in different reports, but it is apparent that they occur as a carrier state or low grade infection and are spread to other rodents, including mice, in which the streptobacillus has produced an epizootic disease with lesions in the joints and heart, leading to erroneous comparisons with rheumatic fever.

Brown and Nunemaker[6] suggest that the streptobacillus causes the disease more often than the spirillum. Wheeler[24] and Dolman, Kerr, Chang and Scherer, on the other hand, believe that the former is the true cause of the disease.

TRANSMISSION

The wild rat is the chief reservoir of infection, which is transmitted to other species of susceptible animals and to man by biting or contact. Human cases have been attributed to the bites of bandicoots, cats, ferrets and weasels. No human insect vectors have been reported, and no human-to-human transmission is known to occur.

Streptobacillus moniliformis, a contaminant in the rat's mouth, is evidently introduced into the wound at the time of the bite. *Spirillum minus*, however, has not been observed in the saliva of rats, and either gains access to the rat's mouth from bleeding gums or from other lesions in the oral cavity. The organism is also in the conjunctival exudate and serves as a source of contamination in the mouth through the lacrimal ducts.

PATHOLOGY

The relatively low incidence of rat-bite fever and a low mortality rate have precluded an extensive knowledge of the pathogenesis and pathology in man. Comparatively few autopsies have been carried out, and often the cases were complicated with secondary infection. Microscopically, the primary lesion is characterized as a nonsuppurating granuloma. The epithelium at the wound site shows necrosis and infiltration of the corium with round cells. The secondary lesions opened as exanthematous patches likewise show round cell infiltration and markedly dilated blood

vessels. The primary bite wound often heals prior to the onset of symptoms, but occasionally an ulcer develops and persists until satisfactory therapy has been initiated. If healing of the primary site takes place promptly, with the onset of general symptoms, the local lesion may become edematous and purplish red. Lymphangitis and lymphadenitis then appear in the area adjacent to the wound. At autopsy, pneumonia, myocarditis and endocarditis are often seen. Degenerative changes occur in the kidneys and liver. Pathology of experimental infection in rats and guinea pigs includes lymphadenitis, splenomegaly and congestion of the liver. In addition, guinea pigs exhibit keratitis and conjunctivitis, suffer loss of hair, become emaciated and die one to two months after inoculation.

CLINICAL PICTURE

The signs and symptoms of the fevers due to the two organisms are similar, even though technically we are concerned with two different diseases. The syndromes produced by both agents have in common intermittent chills and fever, and macular and papular lesions of the skin which occur early in the course of the disease. The incubation periods, however, may be most helpful in distinguishing these diseases clinically, the spirillum form having a longer period of one to four weeks, whereas in the streptobacillus form of the disease the incubation period may only be a matter of hours or days. Because we are dealing with two unrelated diseases in this clinic, the symptomatology will be considered in separate paragraphs. We would like to suggest that the classical form due to *Spirillum minus* be known as Rat-bite fever A, and the septicemic form due to *Streptobacillus moniliformis* be known as Rat-bite fever B.

Rat-Bite Fever A (*Spirillum Minus*)

An incubation period of seven to twenty-eight days may follow a bite by a rat, at which time there is an exacerbation of the original wound site, which becomes painful and indurated, and a chancre-like ulcer develops quickly with the sudden onset of chills and fever. The fever persists for three to five days, when it usually subsides by crisis. The symptoms improve, and the ulcer tends to heal over in the next few days. This cycle of recurring pain in the original bite with ulceration, adenitis, chills and fever may then be repeated. Intermittent or recurrent fevers characterize this form of rat-bite fever as well as a skin rash which may be generalized at some time in the course of the illness. The rash begins as small erythematous patches which spread and develop into large, blotchy maculopapular lesions.

A moderate degree of leukocytosis often is present, but is in no way

diagnostic. The Kahn flocculation test is frequently positive in contrast to the Wassermann complement fixation test, which is usually negative or only weakly positive. Woolley stated that the positive Kahn, Hinton and Mazzini tests are seen exclusively in this A (*Spirillum minus*) form of the disease.

Rat-Bite Fever B (*Streptobacillus Moniliformis*)

The incubation period in this disease may be a few hours to seven days, the average being about five days. The initial wound usually heals and does not have the chancre-like exacerbation characteristic of the first or A form of the disease The fever tends to be septic in type and is not associated with regional adenitis as a rule. Joint pains are commonly observed, and the joints may be red and swollen, and more than one may be involved. The joint may show arthritic changes by roentgenography.

A rash is also present in this disease; it may be petechial, but also may appear as a blotchy maculopapular eruption simulating the erythemas associated with rheumatic fever.

Differential Features

A moderate leukocytosis is usually associated with both diseases and so does not help to differentiate them. The primary features of A are its longer incubation, relapsing fever, regional lymphadenitis, indurating ulcer at the site of the bite and falsely positive Kahn tests. Type B is distinguished by a shorter incubation period, a septic type of fever, some mild swelling of the wound, but usually with prompt healing, arthritis and the serums rarely showing a positive Kahn reaction.

DIAGNOSIS

This must be accomplished bacteriologically. The clinical features may overlap so completely that accurate diagnosis requires the identification of either the spirillum (A) or the streptobacillus (B). This is of course the best way to differentiate the rat-bite fevers from other diseases. It must be kept in mind that both organisms may be involved in the same patient, since the rat may be a carrier of both etiologic agents and infect man with both by its bite.

We might briefly list the important diseases to consider in the differential diagnosis as follows: (1) cat-scratch fever, which produces a regional adenitis and fever, (2) acute infectious arthritis, (3) rheumatic fever, (4) malaria, (5) syphilis, (6) dengue, (7) measles, (8) erythema multiforme, (9) Weil's disease, or leptospirosis icterohemorrhagia, (10) Rocky Mountain spotted fever.

Laboratory Diagnosis

The specific diagnosis of rat-bite fever is dependent upon laboratory procedures designed to demonstrate *Streptobacillus moniliformis* and *Spirillum minus*. The infectious agents are present in the exudates expressed from the tissue surrounding the primary lesion in the patient's blood, joint fluid, serum from secondary exanthematous patches, and in the lymph nodes, especially those draining the infected wound. Thus specimens from these sources must be obtained and examined.

Bacteriology. Blood cultures for *Streptobacillus moniliformis* may be made in tryptose phosphate broth or in trypticase soy broth to which has been added from 10 to 20 per cent ascitic fluid. The media should be inoculated with joint fluid, exudate from lesions, and material obtained from lymph node puncture. Chocolate and blood agar plates should also be inoculated with similar exudates and, if possible, with tissue from a biopsied lymph node. Incubation at 36° to 36.5° C. in an atmosphere of 10 per cent carbon dioxide is essential.

Animal Inoculation. Streptobacillus moniliformis may be readily demonstrated by the inoculation of embryonated eggs and mice with any of the material from patients. Mice die in four to six days after injection. The organism may be obtained from the blood, liver or spleen of mice, from the chorio-allantoic membrane and from the joints of the chick embryo where the infections localize. *Spirillum minus* may be detected by darkfield examination of the blood of inoculated mice and guinea pigs two or three weeks after inoculation. Occasionally the animals show no signs of illness. Animals free from *Spirillum minus* must be used for diagnostic work.

Serology. Agglutination tests with patient's serum usually become positive in two to three weeks after infection. The antigen is available in only a few laboratories, however. Maximum titers are obtained in two to three months after the onset of symptoms. Agglutinins may persist for eighteen to twenty-four months. Falsely positive serologic tests for syphilis are not infrequently observed.

Dermal Sensitivity. Intradermal tests with suitable antigens prepared from *Streptobacillus moniliformis* remain positive for five or six months.

Darkfield Microscopy. Spirillum minus is best demonstrated by darkfield examination of serous exudates from the primary lesion, secondary exanthematous patches, and from the patient's blood. The blood of mice and guinea pigs inoculated with material from patients should likewise be examined by darkfield illumination for the spirillum.

TREATMENT AND PROGNOSIS

These have become greatly simplified with the use of penicillin, which may even have important diagnostic value as a therapeutic test. Both

forms of rat-bite fever respond promptly to penicillin, and the dose to be used can usually be determined by the age of the patient and the severity of the disease. A seven day course is usually recommended unless the complications are marked.

In 1943 Lourie and Collier[16] first reported penicillin to be superior to the use of arsenicals, and Heilman and Herell[14] in 1944 reported on experimental studies in mice in which penicillin was found effective against both the spirillum and the streptobacillus. Robins[20] treated a patient with infection caused by the streptobacillus successfully in 1944 with penicillin, and Wheeler[24] in 1945 treated a patient with a spirillum infection with penicillin for the first time. Many have been treated since, usually with excellent results. Of interest is the report of Weber and Favour,[23] who found the organism very sensitive to penicillin in vitro, but subsequent to further cultivations on artificial media resistant to the drug. Attention should be given to anemia, and transfusions should be given if necessary in severely ill patients.

REFERENCES

1. Allbritten, F. F., Sheely, R. F., and Jeffers, W. A.: Haverhillia Multiformis Septicemia; Its Etiologic and Clinical Relationship to Haverhill and Rat-Bite Fevers. J.A.M.A., *114*:2360, 1940.
2. Altemeier, W. A., Snyder, H., and Howe, G.: Penicillin Therapy in Rat-Bite Fever. J.A.M.A., *127*:270, 1945.
3. Bayne-Jones, S.: Rat Bite Fever in the United States. Internat. Clin., 3:235, 1931.
4. Beeson, P. B.: Problem of Etiology of Rat Bite Fever; Report of Two Cases Due to Spirillum Minus. J.A.M.A., *123*:332, 1943.
5. Blake, F. G., Horstmann, D. M., and Arnold, H.: Fatal Case of Rate-Bite Fever Due to Streptobacillus Moniliformis. Yale J. Biol. & Med., *16*:589, 764, 1944.
6. Brown, T. M., and Nunemaker, J. C.: Rat-Bite Fever; Review of American Cases with Reevaluation of Etiology; Report of Cases. Bull. Johns Hopkins Hosp., *70*:201, 1942.
7. Buddingh, G. J.: Experimental Streptobacillus Moniliformis Arthritis in the Chick Embryo. J. Exper. Med., *80*:59, 1944.
8. Byers, G. A.: Rat Bite Fever. J.A.M.A., *117*:1807, 1941.
9. Edelman, S. D., and Haber, G. B.: Rat-Bite Fever; Report of 3 Cases with Review of Literature. J. Pediat., 5:520, 1934.
10. Futaki, K., Takaki, I., Taniguchi, T., and Osumi, S.: Spirochaeta Morsus Muris, Small n. sp. the Cause of Rat-Bite Fever. J. Exper. Med., 25:33, 1917.
11. Greengard, J., and Hess, E. R.: Rat-Bite Fever in an Infant Bitten at the Age of Eleven Days. J.A.M.A., *116*:2393, 1941.
12. Griffith, R. L., and McNaughton, D. W.: Report of a Case of Rat-Bite Fever Due to Streptobacillus Moniliformis. Pub. Health Rep., 68:947, 1953.
13. Heilman, F. R.: Study of Asterococcus Muris (Streptobacillus Moniliformis); Morphologic Aspects and Nomenclature. J. Infect. Dis., *69*:32, 1941.
14. Heilman, F. R., and Herrell, W. E.: Penicillin in Treatment of Experimental Infections with Spirillum Minus and Streptobacillus Moniliformis (Rat-Bite Fever). Proc. Staff Meet., Mayo Clin., *19*:257, 1944.
15. Levaditi, C., Nicolau, S., and Poincloux, P.: Etiology of Acute Multiform Erythema; Streptobacillus Moniliformis as Its Causal Factor. Presse méd., 34:340, 1926.
16. Lourie, E. M., and Collier, H. O. J.: The Therapeutic Action of Penicillin on

Spirochaeta Recurrentis and Spirillum Minus in Mice. Ann. Trop. Med., 37:200, 1943.
17. Miyake, H.: Ueber die Rattenbisskrankheit. Mitt a. d. Grenzgeb. d. Med. u. Chir., Jena, 5:231, 1899.
18. Richter, C. P.: Incidence of Rat Bites and Rat Bite Fever in Baltimore. J.A.M.A., 128:324, 1945.
19. Robertson, A.: Observations on the Causal Organism of Rat-Bite Fever in Man. Ann. Trop. Med., 18:147, 1924. Spirillum Minus Carter, 1887, the Aetiological Agent of Rat-Bite Fever: A Review. Ann. Trop. Med., 24:367, 1930.
20. Robins, G.: Haverhill Fever Following Rat Bite Treated with Penicillin; Report of Case. Clinics, 3:425, 1944.
21. Sabin, A. B.: Streptobacillus Moniliformis, in Dubos, R. J.: Bacterial and Mycotic Infections of Man. 2d ed. Philadelphia, J. B. Lippincott Company, 1952, p. 563.
22. Watkins, C. G.: Rat-Bite Fever. J. Pediat., 28:429, 1946.
23. Weber, R. A., and Favour, C. B.: Rat Bite Fever Due to Streptobacillus Moniliformis Treated with Penicillin; Case Report. Bull. Johns Hopkins Hosp., 77:132, 1945.
24. Wheeler, W. E.: Treatment of the Rat Bite Fevers with Penicillin. Am. J. Dis. Child., 69:215, 1945.
25. Wilcox, W.: Violent Symptoms from the Bite of a Rat. Am. J. M. Sc., 26:245, 1840.
26. Woolley, P. V., Jr.: Miscellaneous Notes on Rat Bite Fever; Report of 2 Cases: Review of American Reports from 1931 to 1940; Observations on Isolation of Spirillum Minus and Results of Standard Serologic Tests during Rat Bite Fever. J. Pediat., 19:513, 1941.
27. Woolley, P. V., Jr.: Conference at the Children's Hospital of Michigan. J. Pediat., 33:506, 1948.

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COCCIDIOIDOMYCOSIS*

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Coccidioidomycosis can qualify as an *unusual* infection of childhood or of any age in most of the United States. However, in the endemic areas of our arid Southwest, where 70 to 90 per cent of long-time residents have been infected, its incidence is fifty to 500 per 1000 per year[32] and thus is as common a childhood infection as measles or chickenpox. Fortunately, most of these infections are symptomless. A few are progressive and fatal.

The disseminated form was first recognized and described in North America by Rixford[26] in 1894. He and Gilchrist, believing that the etiologic agent resembled the animal parasite Coccidia, named it *Coccidioides immitis*.[27] In 1900 Ophüls and Moffit[24] published and amplified the discovery of Thorne that the organism is a fungus. Until 1936 the infection was noteworthy because of its pathologic and clinical mimicry of extrapulmonary tuberculosis with a fatality rate approximating 50 per cent, because of the diphasic nature of the fungus and because of its geographic restriction. Thus outside California it was known as "the California disease." In California it was more generally termed "the San Joaquin Valley disease." Ophüls[23] had hypothecated that the infection was mainly acquired by inhalation of the vegetative spores. Stewart and Meyer recovered *Coccidioides* from San Joaquin Valley soil.[38] Then in 1936-37, working partly independently and partly in collaboration, Myrnie Gifford[12, 13] and Ernest Dickson[5, 6] proved that *Coccidioides* is the cause of the spectacular and common valley fever, or desert rheumatism, characterized by erythema nodosum or multiforme, often accompanied by severe joint pain and usually preceded by an acute respiratory infection. Though the disease frequently is incapacitating, death (generally by meningitis) is rare. Dickson[5] proposed and there has been gen-

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eral acceptance of the term "coccidiodomycosis" to include all forms of infection by *Coccidioides immitis*. The Gifford-Dickson discovery was prophetically termed by Karl F. Meyer[21] "the Renaissance of our knowledge of Coccidioidomycosis."

Especially significant in broadening our knowledge have been the training operations of the three armed services in the endemic areas. These provided opportunities for controlled studies by a number of alert, capable medical and laboratory officers. The support, first by the Rosenberg Foundation, then by the Armed Forces Epidemiological Board, has enabled the coordination of these studies. Of later years the interest of these doctors returned to civilian life has added vastly to our knowledge of the pathogenesis, epidemiology and diagnosis of coccidioidomycosis.[28] Unfortunately, its treatment is still unsatisfactory.

ETIOLOGY, AND CHARACTERISTICS OF THE AGENT

Coccidioides immitis, though an imperfect fungus, has been classed as a hyphomycete.[2] The "sporangial," "spherule" or "parasitic" form has a doubly refractile capsule and multiplies by endosporulation. No true budding occurs. In tissue sections and in Papanicolaou smears, when these criteria are fulfilled, identification can be made. Cover-slip preparations are unreliable, however, and even in tissue sections errors occur. Sometimes on the surface of pulmonary coccidioidal cavities the mycelial "saprophytic" form may be seen. Otherwise only the sporangia occur in animals and measure 8 to 100 microns in diameter. On artificial media the sporangia sprout hyphae and the mycelia soon become septate. Characteristically, arthospores and sometimes chlamydospores form, so that as a culture dries it may consist solely of myriads of these rectangular or cuboidal spores 1.5 to 10 microns in size. They are well adapted to being wafted through the air either in the endemic areas or in laboratories and, once inhaled, being retained in the pulmonary alveoli. Occasionally they may enter through abrasions, though this is rare.[43] Though some mycologists claim to be able to identify *Coccidioides* merely by gross and microscopic examination of cultures, the strains of the fungus differ greatly, so that the pitfalls are serious.[10] Differential media also are helpful,[11, 30] but in identification there is no substitute for demonstration of the diphasic nature of the fungus by culture and animal inoculation.

How the fungus multiplies in nature still is undetermined. Man, dogs, cattle, sheep, rodents, all are infected in endemic areas. Chickens and presumably birds are resistant to massive inoculation. Though *Histoplasma* is identifiable in nature by its characteristic tuberculate chlamydospores, the spores of *Coccidioides* are nondescript and cannot be identified even in soil from which *Coccidioides* has been recovered ex-

cept by culture and animal inoculation. A solution of ammonium acetate can provide nitrogen and carbon for the growth of the fungus, which also can be grown on vegetables or even in heated moistened soil.

INCIDENCE AND GEOGRAPHIC DISTRIBUTION

In contradistinction to *Histoplasma,* which is found in moist areas, *Coccidioides* is confined to arid and semi-arid regions. Occasional human cases have been reported from the Balkans and even the Orient. How-

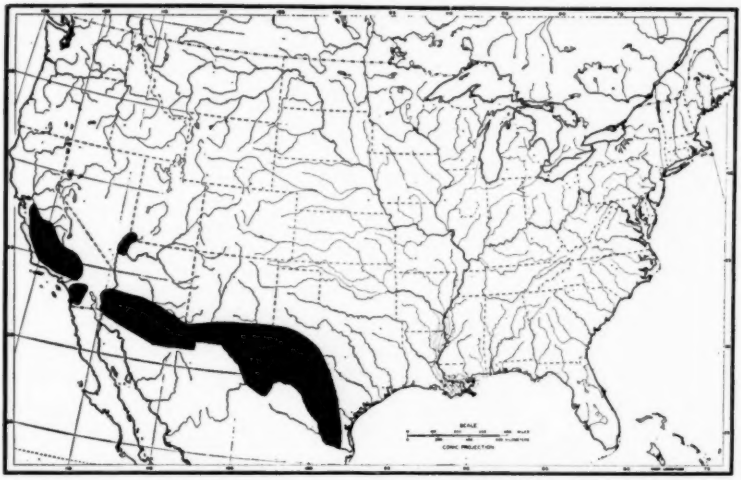

Fig. 9. Known coccidioidal endemic areas in the United States. (Permission to reproduce outline map by University of Chicago Press.)

ever, the only endemic areas which have been established definitely are in the Western Hemisphere. The original cases were reported by Posadas[25] and Wernicke[40] in Argentina. In South America coccidioidomycosis is known as "Posadas' disease."[3] Northward, coccidioidomycosis has been demonstrated in the northern sections of Paraguay[15] and may well extend to the arid areas of Peru and Ecuador, for it is also found in the arid province of Lara in Venezuela.[3] The endemic arid area of northern Mexico adjoins our arid southwestern states of Texas, New Mexico, Arizona and California (Fig. 9). Additionally, the southwestern areas of Utah and Nevada are involved, though lightly.[31] The endemic area in Texas extends all along the Rio Grande River from El Paso to within 100 miles of the Gulf and northward nearly to San Antonio. The histoplasmal area of northern and eastern Texas is free of coccidioidomycosis. The New Mexico area, though ill defined, is in the south and

does not extend north as far as Albuquerque or Santa Fe. As Aronson[1] showed, the northern part of Arizona also appears to be free of the infection. In south central Arizona around Florence, as high as 50 per cent of susceptibles may be infected in six months. California's endemic area is not confined to the San Joaquin Valley. It also includes adjacent slopes over the crest of the Coast Range mountains, the San Fernando Valley of Los Angeles County,[18, 19] spottily[42] in Riverside, San Bernardino and San Diego counties, the gorilla in San Diego's zoo having died of coccidioidal granuloma.[20]

Maximal incidence of infection in the San Joaquin Valley occurs in the dusty summer and fall.[29, 32] Dust control in military installations in the areas of maximal incidence reduced annual incidence among susceptibles from 25 per cent per year to 8 per cent per year.[32] Eventually, however, despite most rigorous dust control measures, virtually all long-time residents will have been infected.

AGE, SEX, RACE

These host factors have no known influence on susceptibility to infection. An infection confers immunity; so increasing prevalence of infection with increasing age merely reflects an association of increasing duration of exposure.

Host factors are important in the clinical manifestations or a spectrum of disease. As yet a critical analysis of the effect of age has not been published. Though erythema nodosum occurs in only 3 to 4 per cent of adult males, this true "valley fever" occurs in 10 to 25 per cent of adult females.[33] Erythema nodosum occurs infrequently in Negroes and Filipinos.[29] However, the dreaded disseminated, progressive disease is much more common in males than in females[13] and occurs at least ten times as often in Negroes and Filipinos[13, 33] as in whites. Mexicans appear to be in an intermediate status.

SOURCE OF INFECTION

The arthrospores and chlamydospores are inhaled and, converting to sporangia, reproduce by endosporulation. The endospores, rupturing the wall of the sporangium, may be carried by the blood stream or lymphatics to distant parts, but generally remain localized. Apparently the spherules are not mechanically adapted to being wafted about, so that no instance of contagion has ever been proved. On the other hand, under exceptional circumstances persons have been infected by the arthrospores carried on clothing or dusty products. Isolation is not necessary or indicated, and in disposal of sputum or dressings from discharging lesions ordinary hygienic precautions will suffice. The sporangia *are* infective, as

a cutaneous infection acquired in a mortuary proved.[43] However, there is no practical problem of direct person-to-person or animal-to-man transmission.

Occasionally, those visiting or even riding by automobile or train through the endemic areas will acquire infections. The incubation period is eight to thirty days, usually ten to sixteen days.

PATHOLOGY

Coccidioidomycosis is one of the infectious granulomas.[9] Indeed, "coccidioidal granuloma" was the designation by the eminent pathologist William Ophüls[23] for the clinical entity we now term "disseminated" or "progressive" coccidioidomycosis and to which this name still clings. In chronic, fibrotic lesions the epithelioid and Langhans giant cells are indistinguishable from those of tuberculosis. The giant cells frequently contain sporangia. In old lesions the organisms may be found only after diligent search. Calcification has been observed both in human and in experimental infections. The same histologic structures are found in focalized solitary pulmonary residuals of the infection. In progressive disease one may find fibrotic and even calcified lesions simultaneously with acute active lesions exhibiting round cell or eosinophilic infiltration and even caseation.[9] These lesions of varying anatomic age have given rise to the belief that dissemination can occur many years after a primary infection. However, in thoroughly focalized infections such late dissemination is extremely rare.

CLINICAL PICTURE

We frequently allude to sharply defined clinical entities in coccidioidal infections, but a merging spectrum is more descriptive.[33] At one end of the spectrum is completely inapparent infection. At the other extreme is the fatal dissemination (coccidioidal granuloma). The manifestations of the hosts' responses pass almost imperceptibly from one extreme to the other. Extensive war-time experiences have given us a reasonably accurate knowledge of this spectrum in adults. Comparable knowledge for children is not available. There is an *impression* that the inapparent infections are more frequent and the clinical course briefer in children. However, there are many severely affected children, with tragic disseminations and all-too-frequent meningitis.

In adults, approximately three fifths of the infections are inapparent, detected only by conversion from a negative to a positive skin test. Of the 40 per cent who have symptoms, there may be only transient malaise, cough or pleural pain, sometimes referred to the neck or shoulder. The patient with maximal signs and symptoms has the following:

Pleural Pain. This may be so severe as to be mistaken for coronary occlusion, cholelithiasis, nephrolithiasis, appendicitis or fractured ribs.

Malaise. The malaise frequently gives rise to a diagnosis of influenza or, occasionally, even typhoid fever.

Fever. Generally diurnal, it usually ranges from 99° to 101° F., but may climb to 104° or 105°.

Cough. Cough, though usually not productive, frequently is irritating and incessant.

Headache. This may be so severe as to be diagnosed as a brain tumor or poliomyelitis.

Backache. This, too, may be severe.

Night Sweats. They may be drenching and require changing of bedding three or four times a night.

Anorexia. The loss of appetite may result in a 30 pound weight loss in an adult. Usually, return of appetite is the first stage in recovery.

Rash. Early in the course a toxic erythema may give rise to a diagnosis of measles or scarlet fever. It should not be confused with the later erythema multiforme.

Sore Throat. Occasionally there is a pharyngitis, but without exudate.

Other Features. Except for the fever and occasional rash, physical examination is usually unrevealing. Even with extensive pulmonary consolidation, physical signs are usually absent, unless fluid also develops. Sometimes massive effusion will completely fill one pleural space.

In from three to twenty-one days, when sensitivity becomes maximal, erythema nodosum with or without erythema multiforme may develop. Prior to puberty there is little evidence of greater predilection for females,[29] but as pointed out previously, in adults erythema nodosum is three to five times more frequent in females. The distribution is usually symmetrical, greatest over the anterior tibial surfaces, frequently on the knees, lateral surfaces of the thighs or buttocks, sometimes on the arms and face.[7] Over the shins it may be confluent. Occasionally an early single lesion has been incised by the unwary. Erythema multiforme is most frequent on the palms, lateral surfaces of the arms and forearms, in a collar distribution around the neck and on the face. Indeed, this form has been mistaken for smallpox. Arthritis occurs frequently at this stage, occasionally posing a differentiation from rheumatic fever. The joints, though painful and tender, are not swollen, nor does hydrarthrosis occur. Sometimes phlyctenular conjunctivitis also develops.

Cavitation

In a few patients (perhaps 0.5 to 3 per cent) rarefaction occurs in the site of one or more of the pulmonary lesions.[34] Cavitation usually de-

velops even while temperature and sedimentation rates are returning to normal and while titers of positive serologic tests are waning. Coccidioidal cavitation may occur some months after all symptoms have ended. *Cavitation occurs in the site of original pneumonitis.* It is not comparable to reinfection type tuberculosis, nor should it be confused with coccidioidal dissemination. Generally there are no signs or symptoms which indicate that cavitation has occurred, although among them the most frequent is hemoptysis. Hemoptysis is seldom severe. Occasionally there is pain or productive cough. In 90 per cent the cavity is single, but sometimes it is multiple or multilocular.[34] Characteristically, it is thin-walled and is more frequent in the upper than in the lower lung field. Radiologists may strongly suspect that a cavity is coccidioidal, but laboratory proof is essential. As has been implied, the prognosis is favorable. Most cavities close rapidly, though some may epithelialize and persist indefinitely.

Disseminated Coccidioidomycosis

In the person who is undergoing an extrapulmonary spread ("dissemination") a single lesion may appear in the skin, bone, a lymph node, meninges, thyroid or gonads. A skin lesion is usually verrucous. The lesions of lymph nodes or subcutaneous tissue appear as cold abscesses and, like osteomyelitic lesions, often develop sinus tracts. If only a *single* lesion develops, unless it is in the meninges, prognosis still is favorable. However, there may be fulminating progression, especially in the dark-skinned races, the body rapidly being riddled with draining lesions. In these cases the cancellous bones of the skull, ribs and ankles seem especially vulnerable. Sometimes a miliary spread occurs. Nearly all organs may be involved. Peritonitis is not uncommon.

The signs and symptoms of the clinical picture of dissemination depend upon the organs or localization. The only sites which one finds in extrapulmonary tuberculosis which disseminated coccidioidomycosis does not reproduce are intestines and the moth-eaten renal calyx of tuberculous nephritis. The mimicry of *progressive primary tuberculosis* is close. Indeed, the pathogenesis seems comparable, too. Usually the extrapulmonary spread occurs during the acute illness of the primary infection. Occasionally it occurs after an inapparent, symptomless primary infection. Sometimes there is latent period of several months or even a year, although longer intervals are exceedingly rare. Thus a well focalized quiescent infection, asymptomatic, in which the patient has a normal sedimentation rate, absent precipitins and absent or low-titered complement fixation virtually never disseminates. However, those patients whose coccidioidal immunity mechanisms are faulty and who once

undergo a dissemination are in entirely different status. Even after an arrest of ten or fifteen years the infections of some may flare up again and cause death.

Coccidioidal Meningitis

Coccidioidal meningitis warrants special comment. It is clinically and by cerebrospinal fluid chemistry and cell count indistinguishable from tuberculous meningitis. This is the most frequently fatal form of white children and adults, often with no other extrapulmonary involvement. Though recovery may occur in all other forms of dissemination, interference with cerebrospinal drainage eventually kills through internal hydrocephalus even though the infectious process is arrested. Currently there are two patients who have been in this precarious state over a decade after their initial infection and almost immediate development of meningitis. Most patients with coccidioidal meningitis succumb within a few weeks or months.

LABORATORY DIAGNOSIS

As already indicated, the clinical picture and epidemiologic circumstances may make one suspect a coccidioidal infection, but definite diagnosis requires laboratory confirmation. These steps have been discussed elsewhere,[31] and only the highlights will be indicated here.

Coccidioidin Skin Test[37]

This is the initial procedure. A Berkefeld filtrate of a culture of multiple strains of *Coccidioides immitis* is nonantigenic. It should be done *prior* to serologic tests, for its results are needed as indication for performing the tests and for their interpretation. Generally used intradermally in 1:100 dilution, it will not cause a quiescent infection to flare up. There is no focal reaction, though occasionally there may be an uncomfortable local reaction with vesiculation and rarely the constitutional reactions of fever and malaise. Rarely, during the primary infection, it may precipitate a bout of erythema nodosum or cause an exacerbation of such an attack.

Because patients with coccidioidal erythema nodosum or multiforme ("valley fever") are quite sensitive, it is more comfortable to explore their coccidioidal diagnosis with an initial test of 1:1000 or 1:10,000 dilution. A *negative* 1:100 *coccidioidin test eliminates the possibility of erythema nodosum being coccidioidal,* since erythema nodosum is a manifestation of hypersensitivity. Indeed, the significance of the test is essentially that of the tuberculin test. It is *specific* with the exception of

some cross reactions with other deep mycosis such as histoplasmosis and blastomycosis.[36] There is no cross reaction with actinomycosis, cryptococcosis or, of course, tuberculosis.[1] Care must be taken, however, not to use syringes which have had other biologicals in them unless they have been cleaned with some agent such as dichromate solution. Autoclaving does not suffice. The active principle, principally a polysaccharide complex, is quite stable. Kept refrigerated and uncontaminated, the 1:100 dilution remains stable for many months. The conversion from negative

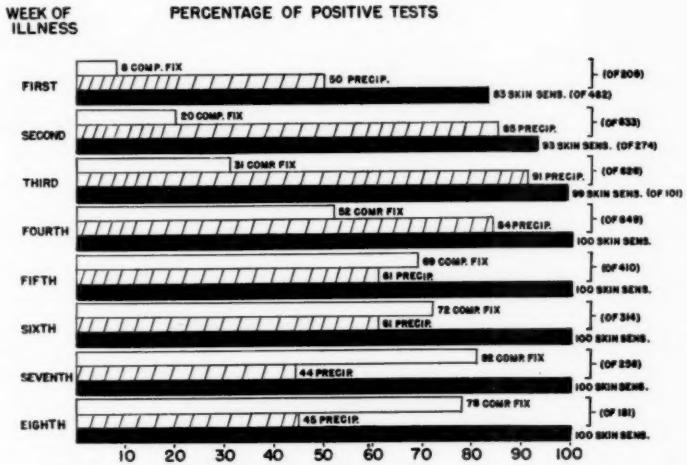

Fig. 10. Percentage distribution of positive precipitin and complement fixation tests in serums and 1:100 coccidioidin tests in skin of patients with primary nondisseminating coccidioidomycosis (by weeks of illness).

to positive skin test is the most accurate and delicate diagnostic procedure and is generally the only proof of asymptomatic infection.[32, 33]

Sensitivity generally is established by the time acute respiratory symptoms develop, but in the first week of symptoms one sixth of the patients still will not react. By the end of four weeks, however, all will react to 1:100 coccidioidin unless dissemination is occurring.[31, 37] Dermal sensitivity antedates the development of the diagnostic humoral antibodies. Therefore there is no point in performing serologic tests for patients undergoing suspected primary infections if the skin test is negative unless dissemination is suspected.[31, 35] These time relationships are indicated in Figure 10. In dissemination the coccidioidin skin test also has prognostic significance.[37] The duration of dermal sensitivity is relatively long, certainly over a number of years and frequently for decades. It probably is not as durable as sensitivity for tuberculin. Sometimes 1:10 coccidoidin is necessary for patients with coccidioidal pulmonary residuals including

cavities, and occasionally these persons may not react even to 1:10 coccidiodin. In a few of these patients their serum will fix complement.

Serologic Tests[35]

Precipitin and complement fixation tests are indicated, depending upon the results of the skin test. Again they are specific with the exception of deep mycoses. While the skin tests are mainly of diagnostic value

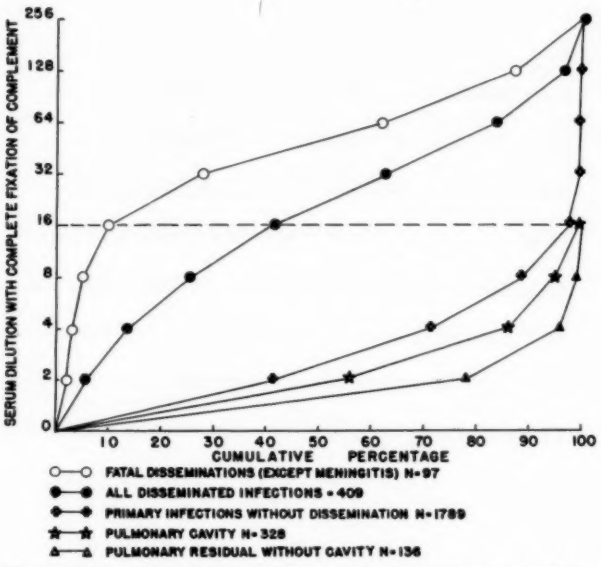

Fig. 11. Maximal titers of complement fixation in serums of various types of coccidioidal disease, expressed as cumulative percentages.

by *exclusion*, the serologic tests are diagnostic by *positivity*, and negative serology does not eliminate the diagnosis except in disseminated infections. In better than 99.5 per cent of such patients serologic tests are positive. In between 90 and 95 per cent of patients with uncomplicated symptomatic primary infections serology is positive, but in only 3 to 7 per cent of patients with asymptomatic primary infections is this true. In only 60 per cent of those with coccidioidal pulmonary cavities can serologic tests establish the diagnosis. Unfortunately, only seldom can serologic tests establish the etiology of asymptomatic coccidioidal pulmonary residuals which are being detected with increased frequency in x-ray surveys. Fortunately, these are not a serious problem for pediatricians, but in older ages when malignancy is a possibility, exploratory

thoracotomy may well be the only means of solving the diagnostic riddle. Then, too, one must be wary lest there be two diagnoses: coccidioidomycosis *and* tuberculosis or malignancy.

Precipitins appear and vanish more quickly than do complement-fixing antibodies. They have little prognostic significance. Complement fixation has an important prognostic use, however. When titers exceed a critical level which is dependent upon the technique used, the outlook becomes ominous for dissemination. The prognostic value of the complement fixation tests is indicated in Figure 11. A progressively rising titer gives cause for alarm, while with a falling titer one can be reassured. Complement-fixing antibodies may persist for years, especially when infection has been severe. The persistence of low-titered complement fixation apparently carries no serious prognostic implication. Serologic tests also may be performed on pleural, ascitic or cerebrospinal fluid. Rarely they may be positive when simultaneous serum specimens are negative. In patients with coccidioidal meningitis repeated cerebrospinal fluid specimens are positive in three fourths, usually one serum dilution lower than in the blood, though occasionally higher. If *complement is fixed* in the cerebrospinal fluid, coccidioidal meningitis is proved. The diagnostic antibodies do not pass the blood-brain barrier.

On the other hand, these antibodies do pass through the placenta, and the titer of cord blood is the same as that of the mother. The titer of the passively transmitted antibodies gradually falls in the infant. A proved case of congenital coccidioidomycosis never has been reported.

Demonstration of *Coccidioides Immitis*

The method of demonstration was discussed under Etiology, combining culture with animal inoculation. Because of the length of time this requires and because of the hazard of laboratory infections, such procedures should be avoided when possible. Therefore serologic tests take first priority. However, especially in coccidioidal cavitation, in which 40 per cent of the diagnoses cannot be established serologically, culturing of sputum or gastric contents is indispensible. We would re-emphasize the unreliability of cover-slip examination. When cerebrospinal fluid cultures are being made, as much fluid as possible should be removed and centrifuged. The supernatant fluid can be used for serologic tests and the sediment cultured.

Hematology

The total leukocyte count is often elevated slightly to 10,000 to 12,000 during the acute phase of the primary infection. An initial polymorphonuclear increase with shift to the left is followed by domi-

nance of lymphocytes during healing. More significant is the frequent eosinophilia, which, while generally 3 to 10 per cent, may occasionally reach 89 per cent.[41]

The sedimentation rate is characteristically elevated during primary infection and disseminated disease. It is one of the most helpful criteria of activity of the process.

Roentgenograms

In primary infection roentgenographic lesions may be demonstrable in symptomless infection and not seen in clinical disease. The lesions may be hilar enlargements, single or multiple parenchymal lesions, nummular or fanning out to involve an entire lobe.[17, 39] They can resemble primary atypical pneumonia, though generally resolving more slowly. They also mimic primary tuberculosis. Residual lesions may persist with or without cavitation and be confused wth tuberculosis or cancer. Occasionally multiple discrete lesions may resemble metastatic malignancy.

Pleural effusion is frequent, generally only small in amount, but occasionally massive.

The thin-walled cystlike cavity of a coccidioidal pulmonary residual is well known. Occasionally the wall is so thin that Lipiodol is necessary for its demonstration. Peripherally located, it can also rupture and produce hydropneumothorax.[34] Spontaneous pneumothorax can complicate a primary infection with no discernible cavity formation. Occasionally, bronchiectasis can be demonstrated secondary to the primary coccidioidal infection.

The roentgenographic appearance of the osseous lesions of disseminated coccidioidomycosis resembles that of tuberculosis, often with considerable new bone formation. Predilection for cancellous bone has been mentioned, and multiple lesions in such sites occurring in a Negro or Filipino male from west Texas, Arizona or the San Joaquin Valley of California should arouse suspicion.

Thus the roentgenographic appearance is not diagnostic, but, like the clinical findings and hematology, beckons the alert to conclusive laboratory measures.

CLINICAL COURSE AND OUTCOME

The primary infection generally is self-limited. When clinical symptoms occur, the length of the illness is variable, ranging from only one or two days to weeks and even months. Illness for one to two months is common. The asthenia may last as long as a year even though dissemination does not occur. This fact is often not appreciated. It holds true for

children as well as for adults. Also pleural pain may persist for several years even though no residual lesion be demonstrable radiographically.

Erythema nodosum may prolong the initial illness because of recurring crops of the lesions. Tenosynovitis and joint pains often persist for several months. However, patients with erythema nodosum are least apt to have dissemination of their infections.

As was previously discussed, coccidioidal pulmonary cavities generally close spontaneously, but may continue indefinitely. Dissemination in patients with pulmonary cavitation is also rare.

Dissemination is comparable to progressive primary tuberculosis and occurs as part of or relatively soon after the primary infection. Death ensues in 50 per cent and is invariable, ultimately, in coccidioidal meningitis. Of course an effective chemotherapeutic agent or antibiotic could change this dismal picture immediately.

CLINICAL MANAGEMENT

The drugs and antibiotics which have been used are legion. Penicillin, streptomycin and the broad-spectrum antibiotics (Terramycin, chloramphenicol, and so on) are valueless. Because of the grim prognosis in disseminating infections, heroic measures with considerable risk have seemed justified. Currently ethyl vanillate is being tried,[4, 8] but it requires prodigious doses which rarely can be tolerated by those with meningitis, whose need is most dire. Thus far the stilbamidines have not been comparable in effectiveness against *Coccidioides* with their success against *Blastomyces*.[16] A difficult problem in evaluating these drugs in man is the variability of the clinical course. Thus several workers were convinced of the clinical efficacy of actidione, although subsequently Ajello[11] showed that it was useful in culture medium to eliminate other fungi and permit isolation of *Coccidioides*.

Of course, trials of many drugs continue, and it is hoped that a satisfactory one will be discovered soon. Until one is discovered, patients with disseminating disease certainly should have the rest and nutritional regimen on which our treatment for tuberculosis exclusively relied prior to the discovery of appropriate antibiotic and chemotherapeutic agents. When a single extrapulmonary cutaneous lesion develops, x-ray therapy and local treatment with thymol in oil or tyrothricin ointment may reduce the size of the lesion. If it persists and can be excised without serious deformity, surgery may shorten the course. Large cold abscesses should be drained, since they will not absorb, and repeated aspirations ultimately result in breaking down of the lesion. Amputation of a singly involved digit or extremity may shorten a course and unquestionably becomes desirable when an artificial extremity is more useful than an extensively involved burdensome limb. Of course there

is hazard of recurrence in the stump of an amputated extremity. Further disseminations do not occur from such lesions, but may continue from the pulmonary site. Arthrodesis of an involved knee or spine may be resorted to as in tuberculosis.

Pulmonary cavities that develop during the course of an acute illness are best treated with rest for a month or so after the toxic symptoms and other criteria to be mentioned have abated. Then if the cavity still persists, the patient should be let up and about with restricted activities, but allowed essentially a normal life. The cavity generally will close. If it continues, however, accompanied by frequent and extensive hemoptyses or repeated bouts of secondary infection, closure should be attempted. Simple measures such as pneumoperitoneum or a phrenic crush may be considered. If these do not suffice, thoracotomy with excision of the cavity by wedge resection or lobectomy almost always will succeed. Occasionally a new cavity or even multiple cavities will occur in overexpanded remaining lung and thoracoplasty may be considered. Conservatism is desirable, recalling that there is no bronchogenic spread from cavities, nor are such patients contagious. Therefore our attitude toward coccidioidal cavitation should differ greatly from that toward tuberculous cavitation.

When a cavity ruptures to the pleural space and hydropneumothorax results, repeated aspirations may be attempted, but only rarely will they eliminate the defect. Therefore, without further temporizing, thoracotomy should be performed, the cavity constituting the bronchopleural fistula excised and the defect repaired and the surface of the lung decorticated. Then the lung will re-expand. These lesions do not disseminate, and the coccidioidal infection is held in check as effectively as though the lesion were tuberculous and the patient being treated with antibiotics.

When diagnosis of a coccidioidal pulmonary residual is made with certainty, one should not tamper with the lesion. If there is question of bronchogenic carcinoma, however, exploratory thoracotomy can be performed with safety, and one should not delay. Fortunately, this is not a problem for pediatricians.

With respect to the primary infection, its management is the key to the treatment of coccidioidomycosis. Without an effective antibiotic, we should minimize the risk of dissemination. Thus the patient should be rested until (1) the temperature returns to normal and other clinical evidence of infection terminates; (2) the sedimentation rate approaches normal; (3) precipitins disappear and complement is fixed only at a low titer or is falling; (4) roentgenographic lesions are regressing.

Unless there seems grave threat of dissemination, lavatory privileges are desirable. With small children the definition of "rest" must be liberal rather than literal; also, the criteria set forth are to be interpreted

intelligently. Occasionally high-titered complement fixation persists even after the sedimentation rate has become normal, or the sedimentation rate may be elevated for many months after all other criteria of focalization have been fulfilled. Under those circumstances it is inappropriate to insist on greatly restricted activities.

When erythema nodosum or multiforme occurs, the antihistaminic drugs are ineffective. Cortisone will control the lesions, but because of the possibility of disseminating the infection, it is rarely resorted to. No case of such a complication has been reported.

PROGNOSIS AND SEQUELAE

Prognosis of the primary infection is excellent. The only lasting complications may be pulmonary cavitation or extrapulmonary dissemination (progressive primary disease). In children these complications are rare, but sometimes occur.

REFERENCES

1. Aronson, J. D., Saylor, R. M., and Parr, E. I.: Relationship of Coccidioidomycosis to Calcified Pulmonary Nodules. Arch. Path., 34:31, 1942.
2. Baker, E. E., Mrak, E. M., and Smith, C. E.: The Morphology, Taxonomy, and Distribution of *Coccidioides Immitis*, Rixford and Gilchrist, 1896. Farlowia, *1*:199, 1943.
3. Campins, H., Scharyj, M., and Gluck, S. V.: Coccidioidomicosis (enfermedad de posadas) su comprobación en Venezuela. Archiv. Venezol. Patol. Trop. y Parasit. Med., *1*:1, 1949.
4. Cohen, R.: Four New Fungicides for *Coccidioides Immitis*: Sodium Caprylate, Ethyl Vanillate, Fracidin, Thiolutin. Arch. Pediat., 68:259, 1951.
5. Dickson, E. C.: Valley Fever. California & West. Med., *47*:151, 1937.
6. Dickson, E. C., and Gifford, M. A.: Coccidioides Infection (Coccidioidomycosis); II. The Primary Type of Infection. Arch. Int. Med., 62:853, 1938.
7. Faber, H. K., Smith, C. E., and Dickson, E. C.: Acute Coccidioidomycosis with Erythema Nodosum in Children. J. Pediat., *15*:163, 1939.
8. Fiese, M. J., Radding, J., Chen, S., and Steinbach, O. K.: Disseminated Coccidioidomycosis. Treatment with Ethyl Vanillate, a Preliminary Report. California Med., *80*:349, 1954.
9. Forbus, W. D., and Bestebreurtje, A. M.: Coccidioidomycosis; A Study of 95 Cases of the Disseminated Type; with Special Reference to the Pathogenesis of the Disease. Mil. Surgeon, *99*:653, 1946.
10. Friedman, L., Pappagianis, D., Berman, R. J., and Smith, C. E.: Studies on *Coccidioides Immitis*. Morphology and Sporulation Capacity of Forty-Seven Strains. J. Lab. & Clin. Med., *42*:438, 1953.
11. Georg, L. K., Ajello, L., and Gordon, M. A.: A Selective Medium for the Isolation of *Coccidioides Immitis*. Science, *114*:387, 1951.
12. Gifford, M. A.: Annual Report Kern County Health Department for the Fiscal Year July 1, 1935, to June 30, 1936, pp. 22–23.
13. Gifford, M. A.: Coccidioidomycosis in Kern County, California. Proceedings of the Sixth Pacific Science Congress of the Pacific Science Association. Berkeley, University of California Press, 1942, Vol. 5, p. 791.
14. Gifford, M. A., Buss, W. C., and Douds, R. J.: Annual Report Kern County

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Health Department for the Fiscal Year July 1, 1936, to June 30, 1937, pp. 39–54.

15. Gomez, R. R.: Endemism of Coccidioidomycosis in the Paraguayan Chaco. California Med., 73:35, 1950.
16. Gordon, L. E., Smith, C. E., Tomkins, M., and Saito, M. T.: Sensitivity of *Coccidioides Immitis* to 2-Hydroxystilbamidine and the Failure of the Drug in the Treatment of Experimental Coccidioidomycosis. J. Lab. & Clin. Med., 43:942, 1954.
17. Jamison, H. W., and Carter, R. A.: The Roentgen Findings in Early Coccidioidomycosis. Radiology, 48:323, 1943.
18. Kessel, J. F., Biddle, M., Tucker, H. A., and Yeaman, A.: The Distribution of Coccidioidomycosis in Southern California. California Med., 73:317, 1950.
19. Kritzer, M. D., Biddle, M., and Kessel, J. F.: An Outbreak of Primary Pulmonary Coccidioidomycosis in Los Angeles County, California. Ann. Int. Med., 33: 960, 1950.
20. McKenney, F. D., Traum, J., and Bonestell, A. E.: Acute Coccidioidomycosis in a Mountain Gorilla (Gorilla Beringeri) with Anatomical Notes. J. Am. Vet. M. A., *104*:136, 1944.
21. Meyer, K. F.: Discussion of paper by Dickson, E. C.: Valley Fever. California & West. Med., 47:155, 1937.
22. Newcomer, V. D., Wright, E. T., Leeb, A. J., Tarbet, J. E., and Sternberg, T. H.: The Evaluation of Nystatin in the Course of Coccidioidomycosis in Mice. J. Invest. Dermat., 22:431, 1954.
23. Ophüls, W.: Further Observations on a Pathogenic Mould Formerly Described as a Protozoan (*Coccidioides Immitis, Coccidioides Pyogenes*). J. Exper. Med., 6:443, 1905.
24. Ophüls, W., and Moffitt, H. C.: A New Pathogenic Mould (Formerly Described as a Protozoan, *Coccidioides Immitis Pyogenes*). Philadelphia Medical Journal, 5:1471, 1900.
25. Posadas, A.: Un nuevo caso de micosis fungoidea con Psorospermias. Ann. Circ. Med. Argentine, 15:585, 1892.
26. Rixford, E.: A Case for Diagnosis Presented before the San Francisco Medico-chirurgical Society, March 5, 1894. Occidental Medical Times, 8:326, 1894.
27. Rixford, E., and Gilchrist, T. C.: Two Cases of Protozoan (Coccidioidal) Infection of the Skin and Other Organs. Johns Hopkins Hosp. Rep., *1*: 209, 1896.
28. Schwarz, J., and Muth, J.: Coccidioidomycosis: A Review. Am. J. M. Sc., 221:89, 1951.
29. Smith, C. E.: The Epidemiology of Acute Coccidioidomycosis with Erythema Nodosum ("San Joaquin" or "Valley Fever"). Am. J. Pub. Health, 30: 600, 1940.
30. Smith, C. E.: Coccidioidomycosis. M. Clin. North America, 27:790, 1943.
31. Smith, C. E.: Diagnosis of Pulmonary Coccidioidomycosis. California Med., 75:385, 1951.
32. Smith, C. E., Beard, R. R., Rosenberger, H. G., and Whiting, E. G.: Effect of Season and Dust Control on Coccidioidomycosis. J.A.M.A., 132:833, 1946.
33. Smith, C. E., Beard, R. R., Whiting, E. G., and Rosenberger, H. G.: Varieties of Coccidioidal Infection in Relation to the Epidemiology and Control of the Diseases. Am. J. Pub. Health, 36:1394, 1946.
34. Smith, C. E., Saito, M. T., and Beard, R. R.: Pathogenesis of Coccidioidomycosis, with Special Reference to Pulmonary Cavitation. Ann. Int. Med., 29:623, 1948.
35. Smith, C. E., and others: Serological Tests in the Diagnosis and Prognosis of Coccidioidomycosis. Am. J. Hyg., 52:1, 1950.
36. Smith, C. E., Saito, M. T., Beard, R. R., Rosenberger, H. G., and Whiting, E. G.: Histoplasmin Sensitivity and Coccidioidal Infection. 1. Occurrence of Cross-Reactions. Am. J. Pub. Health, 39:722, 1949.
37. Smith, C. E., and others: The Use of Coccidioidin. Am. Rev. Tuberc., 57:330, 1948.

38. Stewart, R. A., and Meyer, K. F.: Isolation of *Coccidioides Immitis* (Stiles) from the Soil. Proc. Soc. Exper. Biol. & Med., 29:937, 1932.
39. Sweigert, C. F., Turner, J. W., and Gillespie, J. B.: Clinical and Roentgenological Aspects of Coccidioidomycosis. Am. J. M. Sc., 212:652, 1946.
40. Wernicke, R.: Ueber einen Protozoenbefund bei Mycosis fungoides (?) Centr. Bakt. 12:859, 1892.
41. Willett, F. M., and Oppenheim, E.: Pulmonary Infiltration with Associated Eosinophilia. Am. J. M. Sc., 212:606, 1946
42. Willett, F. M., and Weiss, A.: Coccidioidomycosis in Southern California: Report of a New Endemic Area, with a Review of 100 Cases. Ann. Int. Med., 23:349, 1945.
43. Wilson, J. W., Smith, C. E., and Plunkett, O. A.: Primary Cutaneous Coccidioidomycosis. The Criteria for Diagnosis and a Report of a Case. California Med., 79:233, 1953.

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HISTOPLASMOSIS

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Histoplasmosis is our commonest systemic fungus infection. It is a pleomorphic disease with a wide variety of manifestations, tending in many ways to simulate tuberculosis and often being confused with that infection. It also produces a granulomatous type of tissue reaction, with relatively minimal inflammatory change until advanced stages are reached. Infections vary greatly from relatively inapparent asymptomatic parasitization to overwhelming infection.

Until recent years histoplasmosis was looked upon as a rare disease, invariably progressing to a fatal outcome and of mysterious origin. The disease is still poorly understood by many, and in many instances significant examples are misinterpreted, missed entirely or diagnosed as other processes.

HISTORICAL MARGINALIA

Darling[8] in 1905 observed at autopsy three cases of an infection which had not previously been described. His description included the pathologic findings, the observation of the parasites in the tissues, the pleomorphism of the infection, and something of the range of tissues involved. He interpreted the intracellular parasites as being a protozoon. Subsequently da Rocha Lima,[9] studying Darling's preparations, suggested that the parasite is a yeast.

In 1926 Reilly and Watson[33] described the first North American case.

In 1933 Dodd and Tompkins[11] observed the first case in which an antemortem diagnosis was made.

From the patient described by Dodd and Tompkins, Demonbreun[10] cultivated the parasite and clearly depicted its dual growth character-

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istics. About the same time Hansmann and Schenken[23] observed a case and cultivated the parasite, but did not relate it to Darling's infection.

In 1944 Smith[40] suggested that the examples of nontuberculous pulmonary calcifications, which were observed with great frequency in certain parts of the Midwest and Southeast and which had been described particularly in Tennessee, might be due to histoplasmosis, since infection with this fungus was known to be endemic there from the many case reports of progressive histoplasmosis which came out of the area.

In 1944 Christie and Peterson[4] utilized the histoplasmin test, which had been described previously,[43, 44] to show that many persons living in Tennessee were sensitive to the material, that such sensitivity was associated with known infection, and that many who were sensitive had nontuberculous pulmonary calcification. This remarkable association was promptly verified and amplified by the studies of Palmer.[28] Subsequently the painstaking studies of Furculow[19] and his associates and others traced out in detail the course of the disease and established definitely the relationship between primary pulmonary histoplasmosis and the ultimate pulmonary calcifications.

In 1938 Emmons[14] was able to recover *Histoplasma capsulatum* from soil samples obtained in a run where rats infected with *H. capsulatum* had been trapped. Subsequently, others[17, 22, 45] were able to cultivate *H. capsulatum* from samples of soil taken from areas where people had been infected with *H. capsulatum*, and to show certain factors which favor its growth in the soil. Furculow and his associates were able to obtain soil isolations thought to be significantly related in eleven out of thirteen examples of small epidemics of histoplasmosis.

These and other studies have served to indicate, if not to establish beyond doubt, that histoplasmosis is acquired directly from a contaminated environment, from the free growth of *H. capsulatum* in the soil.

ETIOLOGY

Histoplasmosis is caused by the fungus *Histoplasma capsulatum*. It grows in two forms according to its environment. Free in nature, in certain enriched soils, particularly in those fertilized with bird excreta, and covered or protected, as in sheds or under steps, it grows in a mycelial form, producing a cottony white mycelial mass with hyphae, microconidia and the characteristic tuberculate macroconidia. These features are shown in Figure 12. The tuberculate macroconidium is the identifying mark of the parasite.[10] No other fungi are known to have macroconidia which might be confused with those of histoplasma, particularly no other pathogenic fungi. Some strains of *H. capsulatum*

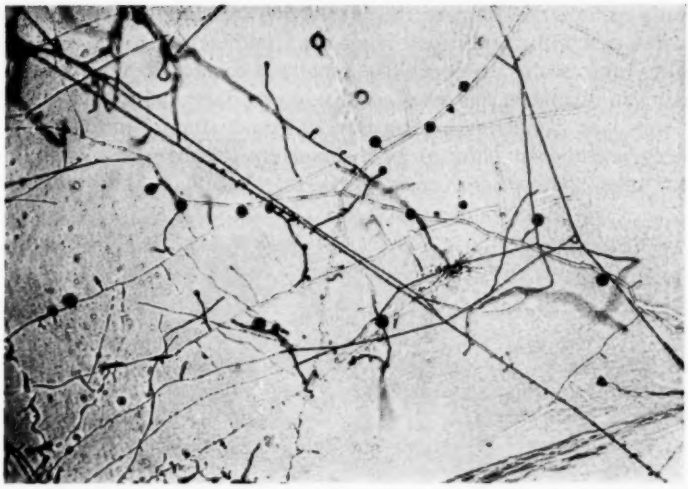

Fig. 12. The mycelial phase of *H. capsulatum*, showing the septate hyphae, the microconidia and the tuberculate macroconidia.

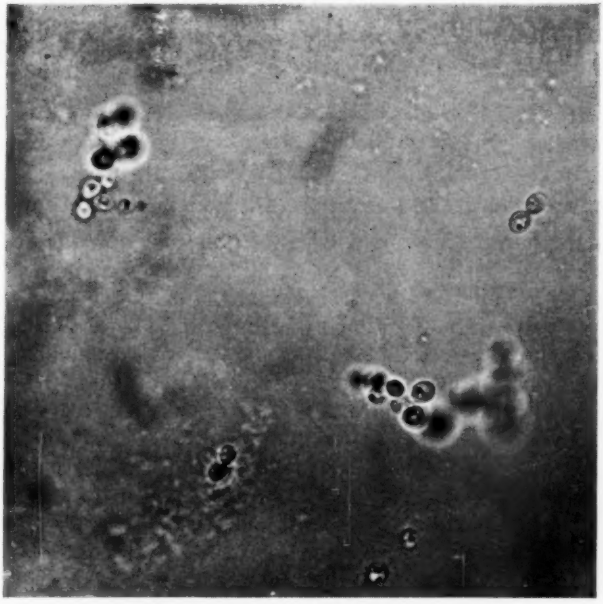

Fig. 13. The yeast phase of *Histoplasma capsulatum*.

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in early cultures do not show this identifying structure and may require repeated subculture and expert study for identification.

The tuberculate macroconidia usually measure about 10 to 18 microns in diameter. The small, relatively undifferentiated microconidia are only 1 or 2 microns in diameter. Different strains from American sources have slightly different gross characteristics, some developing few if any tuberculate macroconidia in the mycelial phase. They also vary

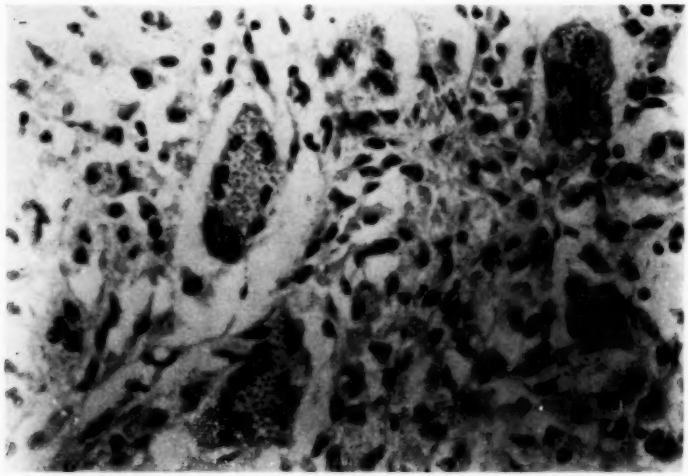

Fig. 14. Large multinuclear cells filled with phagocytosed and proliferating yeast cells. From a section of lung (× 500).

as to the potency of the antigenic components developed on growth, such as the histoplasmin, used both for skin testing and for fixation studies.

In tissues of animal hosts, and when grown on certain forms of enriched culture media at 37° C., the fungus grows as a yeast cell, usually 3 to 5 microns in diameter. In microscopic sections with ordinary stains the yeast cell has a definite border, a capsule, and at one pole or the other of the slightly oval cell there is a crescent of stained cytoplasm.

Most American mycologists have thought that there was only a single species of histoplasma and that all strains yielded the same sensitization. Recently strains isolated in Africa have been thought to be a distinct species of histoplasma and have been termed *Histoplasma dubosii*.[42] The clinical reports of infection with this organism[12, 42] also tend to support the view that it is distinct from the American strain. Most of the cases reported have been examples of skin infections with discoid, infiltrated, raised lesions like large circinate syphilids. Other distinctive character-

istics of these strains are that they show in tissues and on culture large yeast forms, 15 microns or greater in diameter, and encapsulated accumulations of yeast cells. However, in cultures at room temperature the mycelial phase produces characteristic tuberculate macroconidia. This production of large yeast cells and encapsulated aggregates does not serve to set them aside as a separate species, since such forms have been observed in American strains of histoplasma.[32, 36] Further study of the African strains is needed to determine whether they represent a distinct species or merely a strain variation.

Though *H. capsulatum* is not a fastidious organism, growth of the mycelial phase occurring on such poor media as Sabouraud's dextrose media, nevertheless growth is much easier and much more certain when a more complete medium enriched with blood or plasma is used.[25] Certainly it is important to use a good grade of infusion broth with plasma or serum enrichment as the basic medium for primary isolation of the parasites from patients. The better the media, the better the chance to obtain growth from small inocula.

SOURCES OF INFECTION

Analyses of the data on the age at which children in an endemic area acquire sensitivity suggested that histoplasmosis was an infection not commonly acquired within the household, but one which a child acquired with increasing prevalence as soon as he was able to leave the house.

Early studies indicating that dogs were also affected by this parasite naturally suggested that man might acquire the infection from his association with dogs, but epidemiologic studies with skin testing failed to substantiate this theory. It became apparent that not only dogs, but other animals—rats, cats, skunks, horses, cows and possibly others as well—are naturally infected by *H. capsulatum*. Sensitivity tests carried out on cattle and other domesticated animals indicated that it is probable that these animals acquire their infections parallel with man rather than as a result of their association with man, or vice versa.

The cultivation of *H. capsulatum* repeatedly from the soil, and especially in areas related to epidemic infections, the growth of the fungus from air samples in such areas, the studies in sensitization to histoplasmin and other factors make it appear fairly certain that man acquires his infection directly from the growth of the fungus in the soil. It is not known whether it is the macroconidia or the microconidia which are the infectious agents, but it has been shown that the macroconidia can be highly infectious for experimental animals. These findings seem to have removed all the mystery of the source of the infection with histoplasma.

On the other hand, epidemiologic observation and studies indicate that there is relatively little danger of the infection being transmitted directly from person to person. No cases have been reported in which it was thought that one person acquired the infection from another infected person.

PATHOLOGY

Histoplasmosis is characterized pathologically by the formation of granulomatous lesions[29] which are often confused with those of tuberculosis.

The parasites tend to proliferate in the large phagocytic cells of the reticuloendothelial system[1] in various tissues. They multiply till the cells are literally filled with yeast cells, and on rupture the yeasts are phagocytosed by other large mononuclear cells to initiate a new cycle. This

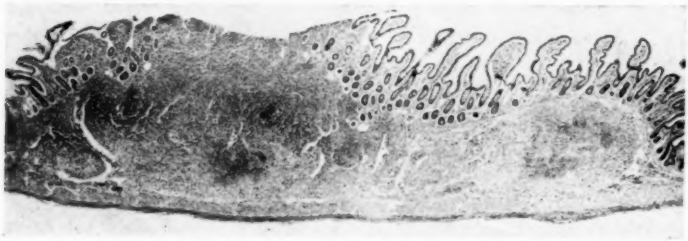

Fig. 15. Lymphoid hyperplasia in a section of the small intestine, due to massive proliferation of large mononuclear cells, filled with yeast. There is ulceration of the mucosa.

reaction goes on with relatively little inflammatory response adjacent in the tissues, until the process is far advanced. These foci tend to become ringed with giant, multinucleated epithelioid cells and may go on to central caseous necrosis. In infants the progressive form of disease is characterized by massive enlargement of the liver and spleen due to such accumulations. The lungs may be similarly involved, and at times the widespread involvement of the pulmonary tissues produces a picture much like that seen in miliary tuberculosis, except that there is a tendency for individual lesions to be larger and more discreet.

The bone marrow is also commonly invaded, and there the proliferation of large mononuclear cells filled with yeast cells may crowd out the normal bone marrow elements to a point at which there is an inability to maintain normal cell maturation. This results in severe anemia and at times leukopenia and thrombocytopenia.

Chronic granulomatous ulcerations of the mouth and hypopharynx occur in about one third of the fatal cases,[39] principally in adults.

Ulcerative lesions may also occur throughout the length of the small intestine and colon. Such lesions are often associated with massive enlargement of the adjacent mesenteric lymph nodes.

The invasion of the adrenal gland often results in marked enlargement of these glands and the development of caseous necrosis of the gland. Such lesions may be associated with adrenal failure and development of signs and symptoms of Addison's disease. This situation is seen more commonly in older patients, but may also occur in infants and children.

Aside from the invasion of the bone marrow, the osseous system is rarely involved, though destructive bone lesions have been described and suppuration of the knee joint has been observed.

The central nervous system is not commonly involved,[38] but evidence of meningitis and of fairly extensive cerebritis has been observed at autopsies, and in one or two instances meningitis has been observed during life.

Involvement of regional lymph nodes may occur in some patients, but marked involvement of the lymph nodes generally is not a common feature of histoplasmosis, even in the generalized disseminated forms. It is true that small, shotty lymph nodes may be observed in most areas, but such involvement is not apt to be an outstanding feature of the disease.

DISTRIBUTION AND PREVALENCE

Our information concerning the distribution and prevalence of histoplasmosis comes primarily from two sources: first, from case reports, mainly of progressive disseminated histoplasmosis, but also of some of the other forms to be mentioned; second, from skin testing with histoplasmin.

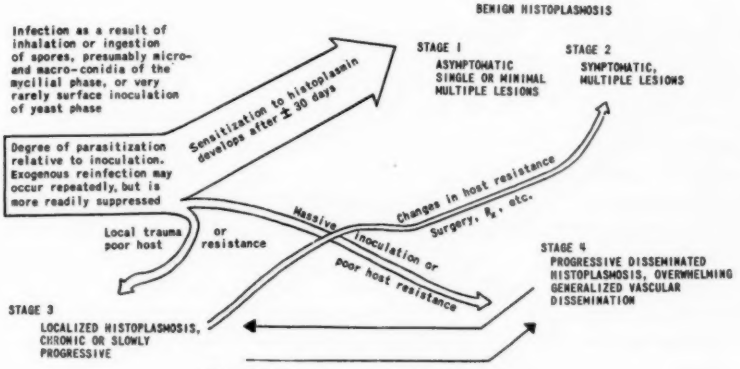
BENIGN HISTOPLASMOSIS
Infection as a result of inhalation or ingestion of spores, presumably micro- and macro-conidia of the mycilial phase, or very rarely surface inoculation of yeast phase
STAGE 1
ASYMPTOMATIC SINGLE OR MINIMAL MULTIPLE LESIONS
STAGE 2
SYMPTOMATIC, MULTIPLE LESIONS
Sensitization to histoplasmin develops after ± 30 days
Degree of parasitization relative to inoculation. Exogenous reinfection may occur repeatedly, but is more readily suppressed
Changes in host resistance
Surgery, R_x, etc.
Massive
Inoculation or
poor host resistance
Local trauma
poor host
or
resistance
STAGE 4
PROGRESSIVE DISSEMINATED HISTOPLASMOSIS, OVERWHELMING GENERALIZED VASCULAR DISSEMINATION
STAGE 3
LOCALIZED HISTOPLASMOSIS, CHRONIC OR SLOWLY PROGRESSIVE

Fig. 16. Spectrum of histoplasmosis.

The majority of the reported cases of histoplasmosis have originated in the central portion of North America.[2, 26] Individual cases, however, and small groups of cases have been reported from areas in North America which are not now looked upon as being endemic areas. These reports indicate minor endemic areas in the Province of Quebec, around Lake Champlain, the Potomac River Valley, some areas in North Carolina, and possibly others.

Darling's cases originated in Panama, and it is only recently that additional case reports have come out of that area, though they must have

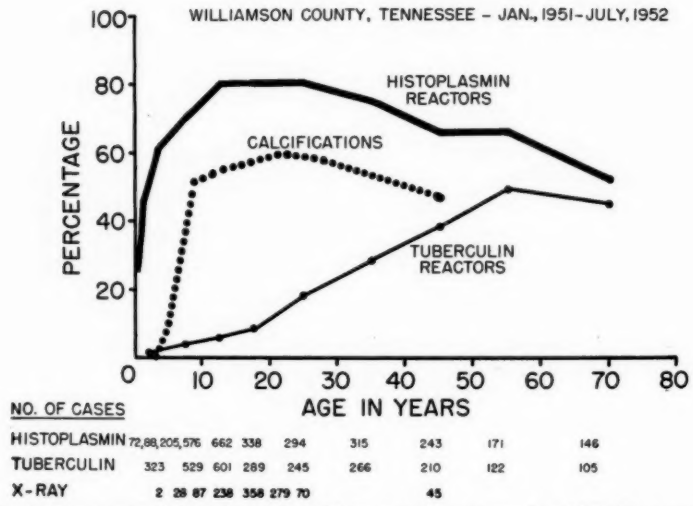

Fig. 17. Prevalence of histoplasmin sensitivity according to age in middle Tennessee, showing its relation to pulmonary calcifications and tuberculin sensitivity.

continued to occur there. Other cases have been reported from Mexico, Honduras, Cuba, Dutch and French Guiana, Brazil, Uruguay, Argentina and Colombia.

Cases have been reported from England, but in each instance they seem to have been importations. The same is true of cases reported from France. However, cases have been reported in life-long residents of Portugal, Spain, Bulgaria, Turkey and Austria. Cases have also been reported from Rhodesia, Belgian Congo and the Union of South Africa. In the Far East, cases have been reported from Indonesia, the Philippines, Java and Australia. A single case has been reported from Hawaii.

Since the introduction of the histoplasmin test[4] there have been many studies which have established its reliability as a tool for epidemiologic observation. In continental North America histoplasmin skin-testing

programs have been carried out in many areas and have mapped out most of the sections where high levels of sensitivity prevail.[2, 26] No histoplasmin sensitivity has been reported from Alaska and northern Canada. Prevalence levels of 10 to 20 per cent sensitization had been reported from some areas around Quebec. In Manitoba and the southwestern provinces of Canada low levels of sensitivity have been observed. In the United States high levels of sensitivity occur throughout the central portion of the great Mississippi Basin, extending from the southwestern portions of Ohio, lower Michigan and most of Illinois and Iowa down through the northern half of Louisiana. In the East the high levels of sensitivity fade out, in eastern Kentucky, West Virginia and Tennessee, but sensitivity is common in parts of North Carolina, Virginia, Pennsylvania and Maryland. In the West high levels of sensitivity prevalence fade out in Kansas and Nebraska. Slightly lower levels of sensitivity prevalence are found in Iowa, northern Illinois, parts of Nebraska, Kansas, Oklahoma and Texas.

Studies in Mexico indicate that the states Chiapas, Nayarit, Sonora, Lower California, San Luis Potosi, and Colima have relatively high levels of sensitivity. Moderately high levels of sensitivity have been reported in Nicaragua and Honduras. In Panama about 38 per cent of the population are reactors, and recently new cases of active progressive histoplasmosis have been reported in that area. Studies from sections of Brazil show levels of sensitivity from 25 to 30 per cent in the eastern part of the Amazon Basin and in southern Brazil. Moderate levels of sensitivity were also observed in Uruguay. In Dutch Guiana and French Guiana studies show levels of 20 to 30 per cent in the younger age group and 40 per cent in persons in the older age groups. Similar studies and levels were found in patients in Cuba, and in recent years cases of active histoplasmosis have been reported from Cuba.

France, England, Denmark, Scandinavia, Ireland, Austria, Spain and The Netherlands all showed low levels or a complete absence of sensitivity.

South Africa, the Belgian Congo and Kenya have sensitivity levels of about 10 per cent.

In India several studies have failed to show significant levels of histoplasmin sensitivity. In upper Burma levels of 14 to 27 per cent positive reactors were encountered, while in lower Burma levels were 4 to 8 per cent, depending on the age group. Histoplasmosis has not been reported from Burma, but x-ray studies have shown fairly typical examples of multiple calcification.

From these data it is apparent that histoplasmosis is worldwide in its distribution, with many areas of high levels of prevalence, and other areas where the disease is relatively uncommon or completely unknown.

Even in areas where sensitivity rates are low the fungus may occa-

sionally cause a recognizable clinical case, but the probabilities of such an occurrence become increasingly less with low levels of sensitivity. It is interesting to note in this relationship that minor epidemics of histoplasmosis have occurred in Wisconsin and Minnesota, areas which have low levels of sensitivity. There persons, exposed to unusual circumstances, are less likely to be protected by previous experience with the infection, just as one can often observe the development of histoplasmosis in persons coming into an endemic area for the first time.

It should also be noted that the persistence of the histoplasmin skin test is not as great as the persistence of the tuberculin test,[46] that histoplasmosis apparently heals to a more complete degree than do tuberculous lesions, with a complete eradication of the yeast cells and a more rapid loss of sensitivity.

CLINICAL MANIFESTATIONS

Histoplasmosis is a disease which varies in the degree of parasitization from a single isolated primary lesion, completely asymptomatic, to one which is progressively disseminated by the blood stream and results in an overwhelming generalized parasitization of the host with marked cachexia and death occurring in a matter of a few weeks. Along this spectrum four types of the disease may be separated. These types are illustrated in Figure 16.

Type I

Histoplasmosis is a benign infection under most circumstances and usually produces lesions of no serious consequence. The common denominator of this type of lesion is the primary pulmonary infection, in which one or two small focal granulomatous lesions develop in the lung, together with a regional lymphadenitis of the hilar glands. These lesions, may, as in tuberculosis, be single primary insertions, but histoplasmosis, in contrast with tuberculosis, is much more commonly associated with multiple areas of involvement as a result of the primary infection, and not uncommonly there may be a great many such lesions even when infection is a benign primary type. This is especially true when the circumstances leading to the development of the infection are the result of heavy contamination, such as that seen in local epidemics of histoplasmosis. In these instances a fairly high percentage of the patients are likely to show multiple pulmonary lesions. However, the lesions are more likely to be single or small multiples of the single insertions, especially in childhood.

For the most part these lesions, once established, go on to heal without the development of recognizable symptoms. Probably such infections

cause minor illnesses which remain either unrecognized or undiagnosed. It is interesting to point out that milder primary infections occurring in laboratory personnel,[18, 27] where the inoculum may be relatively large, are likely to be associated with definite if not severe signs of infection, and symptoms may persist for several weeks before the person feels entirely well again. In some instances such symptoms might be ignored if the patient is not being scrutinized. Whether primary infection is always associated with significant morbidity is a problem which can be solved only by careful clinical examination of patients during the primary infection, and remains for future studies to elucidate.

Type II

A second group emerging from this spectrum is a group in which the infection is of greater severity. Such patients are represented by the

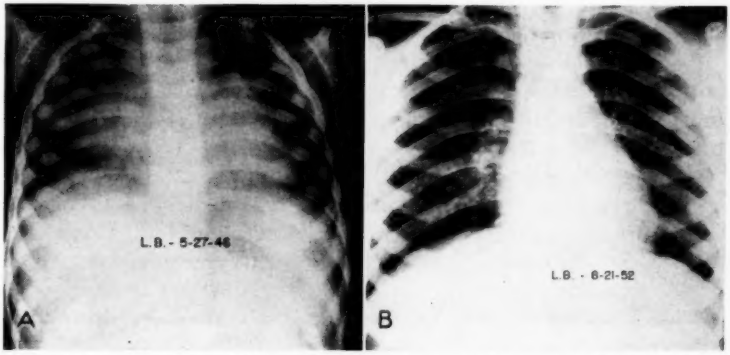

Fig. 18. A case of type II histoplasmosis, primary infection of the lung and hilar lymph nodes. A, The acute phase, showing a diffuse infiltrative process involving both lungs. *B*, The healed phase, showing multiple, calcified pulmonary and hilar lymph node lesions.

L.B., a 9 year old girl admitted because of fever, 100° to 101° F., night sweats, cough and malaise of 2 weeks' duration. The physical examination did not reveal any significant findings. A, The infiltration seen at first examination. The O.T. 0.1 mg. was negative, and the histoplasmin test was 4+. With general supportive care she became fever free in a week and generally improved. Roentgenograms made at intervals showed healing of the pulmonary infiltrations with eventual calcification, as illustrated in *B*.

more severe cases occurring in laboratory workers[18, 27] who have been observed to have definite illness, but even more exemplary are many of the cases in the groups from naturally occurring epidemics in which some of the patients are more severely ill. In many instances these cases may have some degree of dissemination of the infection beyond the primary lymph nodes, but the host reaction is sufficient to overcome the infection. There may be symptoms and signs of the infection last-

ing for weeks to months before the patient finally overcomes the parasite and the infection begins to regress. Individual lesions rarely go on to the point of extensive breakdown, and ulcerative lesions of the alimentary tract do not appear. Such cases are documented[17, 21] in such studies as the group of soldiers who contracted histoplasmosis at Camp Groober while bivouacking in a cellar, the cases described by Sabin and Feldman in which a group of workmen came down with a subacute febrile illness after cleaning pigeon excreta from a water tank, or more certainly by the farm family studied by Loosli and his associates[24] when several people got histoplasmosis from cleaning out a silo.

If one can interpret the relative mortality rates[17, 21] in such reported groups as being indicative of the likelihood of patients having a moderately severe histoplasmosis and surviving, then it would seem probable that many patients have moderately severe histoplasmosis and go on to recovery. Inasmuch as large numbers of these patients have not been reported in the literature, it would seem probable that they are now largely missed, going unstudied and unrecognized, or the process is attributed to other etiologic factors.

The separation of type II from type I is an arbitrary one based primarily on extensiveness of the infection and the severity of the symptoms. Fundamentally, those who have any degree of vascular dissemination would fall into type II.

Ordinarily types I and II go on to complete uneventful healing of the lesions, but there have been a few cases of progressive disseminated histoplasmosis from the breaking down of a healing focal lesion. Such cases must represent an unusual circumstance, equivalent to, but occurring less commonly than, the analogous situation in which a primary tuberculous focus in a school child breaks down to yield miliary tuberculosis.

Type III

The third group of histoplasmosis cases are those in which local lesions develop. The patients from the literature and from our experience who fall into this group have had chronic lesions which show slow, progressive extension which becomes disseminated from the local area and progresses into type IV. Such lesions occur much more commonly in adults, but a few definite cases have been described in childhood.[30]

The tissues most commonly involved in type III involvement are the oropharynx,[39] the larynx and the gastrointestinal tract,[37] all with indolent ulcerative lesions; the lungs with necrosis and cavitation; the adrenals with caseous necrosis; and, less commonly, other tissues such as the central nervous system,[38] the genitalia, the osseous system, and

the skin, which may show ulcerations, papillomatous infiltrations or even tumor-like indurations.

Cases with strict localization to a single tissue are relatively rare. In most instances there is a tendency for the infection to spread, and terminally there is general dissemination as a rule.

In many instances the local lesion heals, with the aid of resective surgery in the case of pulmonary lesions, sometimes oral ulcers and skin lesions with general supportive measures, and sometimes with therapy directed intensively at the local lesions.

Type IV

Though general dissemination of the infection with a fatal outcome may well be the least common type of histoplasmosis, it is nevertheless the one which has been studied most and has been most commonly reported.

Fig. 19. A case of disseminated histoplasmosis.

J.B.W. was admitted to Vanderbilt University Hospital on June 2, 1950, with complaints of cough, fever and irritability of 8 weeks' duration. He had apparently responded to penicillin early in the course, but later the drug and sulfonamides did not affect the course when he was admitted to another hospital. On examination he appeared lethargic and febrile, but in no real distress. There were scattered petechial hemorrhages and some small ecchymotic areas. Small shotty glands were present in the cervical and inguinal areas. The liver and spleen were enlarged. Laboratory studies revealed: red blood cells, 3.4 million; hemoglobin, 9.0 gm.; white blood cells, 20,000, with an essentially normal differential, platelets 56,000, P. C. V., 34, E. S. R., 10; clot retraction, 30 minutes; clotting time, 3 minutes, bleeding time, 5 minutes. Histoplasmin complement fixation test positive 1:40. Histoplasmin 1:100 negative. O. T. 0.1 mg. negative. Bone marrow showed no gross abnormalities, and no parasitized cells were seen, but a culture yielded a growth of *H. capsulatum*; also the blood culture was repeatedly positive for the fungus. In the hospital the patient ran a progressively downhill course, though he was started on therapy with ethyl vanillate 8 days after admission. Roentgenograms of his chest showed marked involvement of the left lung and finally a massive miliary spread throughout both lungs. The complement fixation test remained positive, and on one occasion the titer was 1:80. He died 15 weeks after the onset. A postmortem examination was not done.

General dissemination of histoplasmosis occurs in any age group, but most commonly in two groups, those less than two years of age and those in the fifth decade or older. In the younger group the sex incidence is about the same; in groups beyond childhood, males are affected

Fig. 20. Gross pulmonary lesions in a patient with type IV histoplasmosis, showing diffuse parenchymal changes, involvement of the pleura and extension to the hilar lymph nodes.

much more commonly than females, except in the third decade—the period of childbearing—when incidence in females is as high as in males.

Type IV histoplasmosis, as indicated, may result from the extension of localized infection, but in most instances, especially in childhood, is more likely to be a generalized invasion due to the host's failure to localize the primary infection, either as a result of poor capacity of the host to respond, as in early infancy, or because the inoculation is massive. Presumably the organisms enter either the respiratory or the alimentary tract, and from these sites they are blood-borne to most of the tissues of the body. An example of type IV infection in Figure 19 shows how miliary granulomas have produced tremendous enlargement of the spleen and liver. Such patients commonly show dissemination throughout the lungs, lymph nodes, particularly in the thorax and the abdominal cavity, the bone marrow, the gastrointestinal tract, the liver and spleen,

the adrenals, the kidney and the thymus. To a less extent the heart, skin, lymph nodes generally, and nervous system may be involved.

Such infections are entirely comparable with severe forms of miliary tuberculosis and may be looked upon as having a more serious import now that effective therapy is available for the tuberculous infection. It is true that not all cases of generally disseminated histoplasmosis go on to a fatal termination, but it is equally true that the same situation holds in vascular dissemination of tuberculosis. One must, however, grant that generalized dissemination of histoplasmosis is a less severe infection than generalized dissemination of tuberculosis, just as the local lesions and minor lesions of histoplasmosis carry a less grave prognostic import than do their tuberculous counterparts.

Patients with progressive disseminated infection usually present themselves only in an advanced, often in a cachectic, state. The presenting symptoms can be summarized in the following outline in their relative order of frequency.

1. Fever
2. Abdominal enlargement
   a. Hepatomegaly
   b. Splenomegaly
3. Cough
4. Weight loss
5. Diarrhea
6. Pallor
7. Vomiting
8. Dyspnea
9. Purpura
10. Oral ulcers

On examination the patients commonly appear to have pseudoleukemia, pallor, wasting, hepatosplenomegaly and, in older children, lymphadenopathy. This picture is suggestive enough that experienced interns may strongly suspect the diagnosis from the clinical appearance.

Most infants with this picture, particularly if they are cachectic and showing purpuric manifestations, run a rapidly downhill course and expire in a few days to a few weeks. In rare instances transfusions and supportive measures may result in immediate improvement and change in the downhill progression to one of steady improvement.

LABORATORY DIAGNOSIS

Cultural Identification of Parasites. The diagnosis of histoplasmosis is not difficult to make in most patients with progressive disease. The fungus can usually be cultivated from the sputum in pulmonary cases with progressive destruction of tissue, from the bone marrow or the blood in patients with generalized dissemination, from regional lymph nodes or other biopsy materials or from spleen and liver puncture cultures. The cultures should be carried out with relatively good infusion media enriched with blood proteins and incubated at 20° C. (room temperature). Parallel cultures may be incubated at 37° C. for yeast phase growth. When the materials are grown on plates, it is essential

that the plates be sealed in part to prevent drying, but not enough to occlude oxygen. Parafilm is ideal for this purpose. Growth may be observed on cultures as early as the third or fourth day or as late as the third to fourth week in exceptional cases, usually by the seventh to the ninth day. The cultivation of the fungus and the demonstration of the tuberculate macroconidia are essential to the absolute diagnosis of histoplasmosis.

The second most reliable diagnostic procedure is the demonstration in tissues of specific yeast cell accumulations in the macrophages. That such studies can lead to error is apparent from the fact that no one has been able to confirm earlier studies showing the common presence of histoplasma in the appendices of children. On the other hand, the skilled microscopists can in most instances establish the diagnosis of histoplasmosis with reliable accuracy. Many times the histologic establishment of the diagnosis is missed by a failure of the microscopist to look for the parasites under the higher magnifications.

Histoplasmin Skin Test. Another test used in establishing diagnosis of histoplasmosis is the histoplasmin skin test. Persons who have had other fungus infections, blastomycosis and coccidioidomycosis, commonly react to histoplasmin.[15] However, blastomycosis is a rare disease in children in most parts of the world, and coccidioidomycocis is limited to certain geographic areas which are not coincident with areas of prevalence of histoplasmosis. Furthermore, differential skin testing will usually distinguish such cross reactions. On the other hand, the circumstances under which the presence of a positive histoplasmin skin test can have diagnostic significance are relatively limited, limited to those persons known to be histoplasmin converters. In such instances, if blastomycosis and coccidioidomycosis can be excluded, the probability of the diagnosis of histoplasmosis becomes great. However, in endemic areas the general prevalence of histoplasmin sensitivity among the population precludes the possibility of using this test for diagnostic purposes, except that it is reliable as an exclusion test. Active cases, except in those patients dying from progressive disseminated histoplasmosis, almost invariably react positively. In cases of progressive disseminated histoplasmosis when the patient is in a subterminal phase the incidence of positive reactions to histoplasmin is less than 50 per cent.

Serologic Tests. In addition to the skin test and cultural identification of the parasites, a number of serologic tests for histoplasmosis have been developed. The histoplasmin *complement fixation test*[16, 34] has been used most widely and is valuable for following the course of histoplasma infection. Different fractions of the mycelial antigenic complex and yeast cell preparations have different sensitivities, and no standardized, well established complement fixation test is available at

present. In general, it can be said that complement-fixing antibodies occur in infections of moderate and minimal severity in a high percentage of the cases;[5] they rise early in the course of the disease and fade after a period of a few weeks to months. The antibody response measured by the various modifications of the complement fixation test is relative to the degree of infection.

In addition to the complement-fixing antibody group, an *agglutinating antibody test* has been developed using collodion particles coated with histoplasmin. The usefulness of this test seems limited, since it has not won widespread recognition.

Precipitin tests have also been developed[35] for use in case studies of histoplasmosis. The reaction to the precipitant reaction is developed earlier and lasts for a briefer time during the acute infection than does the complement fixation reaction. However, it is developed with greater regularity in minimal infections than in the complement-fixing reaction, if one can judge from the limited reports available.

In chronic and progressive cases the levels of histoplasmin precipitins and complement-fixing antibodies run roughly parallel, though the titer of precipitin tests may be somewhat higher, particularly early in the disease.

Unfortunately, serologic tests in histoplasmosis lack specificity and are not as reliable as other methods for establishing the presence of infection.

The diagnosis of minimal infection with histoplasmosis is associated with the same difficulties as exact diagnosis in primary tuberculous infections. The presence of a positive skin test establishes the fact that the person has been infected, if coccidioidomycosis and blastomycosis can be excluded, but does not tell us anything about the degree or the stage of the infection. Antibodies capable of complement fixation and precipitation of the histoplasma antigens are found during the acute phase of minimal infection and for relatively long periods of time with chronic and progressive infections, but in terminal states these antibodies may disappear.

CLINICAL DIAGNOSIS

In endemic areas the diagnosis of histoplasmosis should be suspected in infants and young children whenever the question of growth arrest and failure to thrive occurs, especially in conjunction with marked enlargement of the liver and spleen with chronic inexplicable pulmonary disease, with or without the association of leukopenia and anemia. The presence of lesions in the oropharynx and of a gastroenteritis are complementing observations. In adult cases, and in later childhood to a less extent, involvement of the adrenal glands with signs and symp-

toms of adrenal failure is common and should make one suspect histoplasmosis. In the severe forms of the infection the diagnosis can usually be established by cultural techniques.

TREATMENT

Careful attention must be given to a complete and thorough evaluation of the case. Obviously an infection which occurs in this country in endemic areas at a rate high enough that 80 per cent of children have been infected by the age of fifteen years, and in many instances there has been repeated reinfection, must require treatment under only the most unusual circumstances. On the other hand, if the child has a serious progressive process with weight loss, enlargement of the liver and spleen, pulmonary infiltration, anemia, organisms that can be demonstrated on smear or culture from the bone marrow or other tissues, and if the child does not respond promptly to such supportive measures as transfusions, rest and general improvement in the nutritional state, one should consider the possibility of using any available therapeutic agent, even though it may not be an ideal drug.

Two drugs hold some possible claim to effectiveness in the treatment of type IV histoplasmosis.

Ethyl vanillate[3] may be administered in doses beginning at a level of 0.5 gm. per kilogram of body weight per day, increasing gradually, if tolerated and if blood levels do not exceed 20 to 30 mg. per 100 ml., to doses as high as 1.5 gm. per kilogram of body weight per day in infants and children. Severe toxic reactions with nephritis and further liver injury are likely to occur if blood levels greater than 30 mg. per 100 ml. are attained, and levels greater than 20 mg. are essential for the effective treatment of most strains of *H. capsulatum*. It is, of course, apparent that such a drug should be used only as an ultimate resort. On the other hand, it must be administered before the patient becomes cachectic if one is to hope for success.

The second drug which has been used in the treatment of histoplasmosis and which may be effective is Atabrine.[6] Reports of the successful treatment of human histoplasmosis with this drug have not appeared in the literature, but studies have indicated that the drug is effective in the treatment of infected mice if given relatively early in the course of the infection, but not if the mice are seriously ill when therapy is started. Atabrine has also been found to cause the disappearance of *H. capsulatum* from tissues and fluids where it had previously been cultivated even though the disease went on to a fatal termination.[31] Such failures need not entirely condemn the drug, since many patients in the late phases of histoplasmosis are in such poor physical state that they have little or no capacity to respond to treatment.

A third drug which has been reported to have some effectiveness in the topical treatment of histoplasmosis is Propamidine or hydroxystilbamidine.[13] This drug has been successfully used as a topical application in the treatment of ulcers due to histoplasmosis, which had failed to respond to ethyl vanillate, though that drug had been successful in arresting the generalized infection, and it has been demonstrated in vitro to be an effective inhibitor of the organism.

The use of all three drugs in the treatment of patients with histoplasmosis should be looked upon as a highly experimental procedure and should not be undertaken in those patients who do not show unequivocal signs of progressive histoplasmosis. Even those patients who may be symptomatic from moderate infections with histoplasmosis, such as pulmonary infiltration with fever and malaise, should not be looked upon as candidates for such therapeutic procedures.

All other chemotherapeutic and antibiotic drugs which have been introduced to date have been given trials in the treatment of histoplasmosis, without showing any degree of success or promise, though at times the suppression of secondary invaders will promote healing of local ulcerative lesions.

PROGNOSIS

In most instances of histoplasmosis the infection should prove to be a relatively innocuous one which goes on to complete healing after a period of months to a year or so. This, of course, implies that we must lower the clinical horizon for the disease from the level of the generalized disseminated cases to those in stages one and two. The duration of the infection and the persistence of sensitivity to it probably are dependent upon the degree of the infection. In those instances in which the infection is a single minimal primary insertion or has no demonstrable lesion, the lesion may heal completely and the patient may lose his sensitivity in a short time. The calcified nodules taken from the lungs at autopsy are rarely if ever associated with the presence of viable organisms. The loss of histoplasmin sensitivity is fairly great, and during the course of a year's time about 15 per cent of the residents in a community change from positive to negative.[46] During childhood and early adult life the total infection prevalence is so great that there is a constantly rising level of histoplasmin sensitivity. In age groups beyond thirty there is a slow decline in histoplasmin sensitivity, indicating that reinfection does not keep up with loss of sensitivity.

Little is known of the significance of reinfection, or whether such infections are less likely to be progressive than when they occur for the first time, but in all probability the patient with healed lesions has

some degree of immunity and a greater ability to suppress a new infection.

In a few examples necrotic lesions in lungs have broken down and apparently served as a source for the generalized dissemination of the infection. The fact that the great majority of progressive disseminated infections occur in early childhood would suggest that this manner of breakdown and dissemination is not as significant in childhood as is the development of dissemination from massive inoculation. In older persons such lesions might be more important in the development of disseminated infection. There have been numerous cases with symptoms related to chronic ulcerative lesions in the oropharynx, in which there was subsequently a progression of infection to involve the lungs and other tissues. Whether this dissemination occurred at the initiation of the infection or was secondary to the ulcerative lesion in the oropharynx is not known.

Chronic ulcerative forms of the disease of the lungs may remain relatively at a standstill over long periods of time. Some patients with such lesions have been cured by radical surgical excision of diseased tissue.

The question is often asked whether or not there is any danger from the breakdown of lesions after healing to the point of calcification. No cases have been reported which suggest that such lesions may break down and result in dissemination of the infection. In all probability by the time calcium is deposited in the tissues, which may take one to two or three years, the primary granulomatous focus has completely healed, and the yeast cells are all dead.

SUMMARY

Histoplasmosis, our commonest systemic fungus disease, may be looked upon as being a generally benign, often trivial or insignificant infection. In most instances infection results in the development of one or several nodular infiltrates in the lungs, which have a great tendency to heal by calcification after a period of one to three years.

In some instances the infection may be disseminated by the blood stream, and the patient may have considerable morbidity with the infection. In rare instances the dissemination may be massive, and the patient may rapidly lose ground and die after a few weeks. Such severe infections are seen in any period of life, but occur most commonly in the first two years and in the fifth and sixth decades of life.

When dissemination of the infection occurs, many tissues in the body are apt to be affected. Characteristically, invasion occurs in those organs with a large amount of reticuloendothelial tissue, the lungs, the liver,

the spleen, the adrenals and the lymph nodes, especially the lymph nodes regional to the area of primary infection.

In other instances, probably not less common than the progressive disseminated infection, localized processes may develop which remain relatively unchanged over long periods of time.

In most instances the infection gains entry into the body by way of the respiratory tract, but in some instances the primary lesion appears to be in the gastrointestinal tract or even in the oropharynx. The source of the infection is the free existence of the fungus as a saprophyte in the soil.

Treatment of histoplasmosis in most instances is merely supportive and generally symptomatic. In those instances in which lesions are well localized surgical excision may at times be successful, as, for example, the excision of superficial ulcers or in cavitated lesions of the lungs.

Certain diagnosis in histoplasmosis depends upon the cultural identification of the fungus or the pathologic demonstration of characteristic lesions. In other instances it may be strongly suspected when rising titers of histoplasma antibodies can be demonstrated. The skin test, though reliable as a tool for epidemiologic study and as an exclusion test, is of relatively limited diagnostic value in endemic areas.

Histoplasmosis is worldwide in its distribution, but specific geographic localities may be looked upon as being endemic areas with relative degrees of prevalence.

REFERENCES

1. Agress, H., and Gray, S. H.: Histoplasmosis and Reticuloendothelial Hyperplasia. Am. J. Dis. Child., *57*:573, 1939.
2. Christie, A.: Histoplasmosis and Pulmonary Calcification; Geographic Distribution. Am. J. Trop. Med., *31*:742, 1951.
3. Christie, A., Peterson, J. C., Middleton, J. G., and McVickar, D. L.: Treatment of Disseminated Histoplasmosis with Ethyl Vanillate. Pediatrics, *7*:7, 1951.
4. Christie, A., and Peterson, J. C.: Pulmonary Calcifications in Negative Reactors to Tuberculin. Am. J. Pub. Health, *35*:1131, 1945.
5. Campbell, C. C., and Binkley, G. E.: Serologic Diagnosis with Respect to Histoplasmosis, Coccidioidomycosis and Blastomycosis and the Problem of Cross Reaction. J. Lab. & Clin. Med., *42*:896, 1953.
6. Campbell, C. C., and Saslaw, S.: Atabrine Therapy in Histoplasmosis Infection in Mice. Pub. Health Rep., *66*:570, 1951.
7. Clarke, G. H., Walker, J., and Winston, R. M.: African Histoplasmosis. J. Trop. Med. & Hyg. (London), *56*:277, 1953.
8. Darling, S. T.: A Protozoan General Infection Producing Pseudotubercles in the Lungs and Focal Necrosis in the Liver, Spleen and Lymph Nodes. J.A.M.A., *46*:1283, 1906.
9. Da Rocha Lima, H.: Beit zur Kenntnis der Blastomycosin: Lymphangitis epizootica und Histoplasmosis. Centralbl. Bakt., *67*:233, 1913.
10. Demonbreun, W. A.: The Cultivation and Cultural Characteristics of Darling's Histoplasmosis Capsulatum. Am. J. Trop. Med., *14*:93, 1934.

11. Dodd, K., and Tompkins, E. H.: A Case of Histoplasmosis of Darling in an Infant. Am. J. Trop. Med., *14*:127, 1934.
12. Duncan, J. T.: A Unique Form of Histoplasma. Tr. Roy. Soc. Trop. Med. & Hyg., *40*:374, 1947.
13. Ellis, F. F., Scott, R. J., and Miller, J. M.: Treatment of Progressive Disseminated Histoplasmosis with Ethyl Vanillate and Propamidine. Antibiotics, *2*: 347, 1952.
14. Emmons, C. W.: Isolation of *Histoplasma Capsulatum* from Soil. Pub. Health Rep., *64*:892, 1949.
15. Emmons, C. W., Olson, B. J. and Eldridge, W. W.: Studies on the Role of Fungi in Pulmonary Disease. Cross Reactions of Histoplasmin. Pub. Health Rep., *60*:1383, 1945.
16. Furculow, M. L., Bunnell, I. S., and Tennenberg, D. J.: A Complement-Fixation Test for Histoplasmosis. II. Preliminary Results with Human Serum. Pub. Health Rep., *63*:69, 1948.
17. Furculow, M. L., and Grayton, J. T.: Occurrence of Histoplasmosis in Epidemics. Etiological Studies. Am. Rev. Tuberc., *68*:307, 1953.
18. Furculow, M. L., Guntheroth, W. B., and Willis, M. J.: The Frequency of Laboratory Infection with H. Capsulatum. J. Lab. & Clin. Med., *40*:182, 1952.
19. Furculow, M. L., Mantz, H., and Lewis, I.: The Roentgenographic Appearance of Persistent Pulmonary Infiltrations Associated with Sensitivity to Histoplasmin. Pub. Health Rep., *62*:1711, 1947.
20. Furculow, M. L., Willis, M. S., Wood, L. E., and Mantz, H.: Tuberculin and Histoplasmin Conversion Rates in Kansas City as an Indication of Prevalence of Infection—Preliminary Report. B. Histoplasmosis Studies. Tr. Nat. Tuberc. A., *49*:105, 1950.
21. Grayton, J. T., and Furculow, M. L.: Occurrence of Histoplasmosis in Epidemics. Epidemiology Studies. Am. J. Pub. Health, *43*:665, 1953.
22. Grayton, J. T., Loosli, C. G., and Alexander, E. R.: The Isolation of H. Capsulatum from Soil in an Unused Silo. Science, *28*:323, 1951.
23. Hansmann, G. H., and Schenken, J. R.: A Unique Infection in Man Caused by a New Yeast-Like Organism, A Pathogenic Member of the Genus Scpidonium. Am. J. Path., *10*:131, 1934.
24. Loosli, C. G., Grayton, J. T., Alexander, E. R., and Tanzi, F.: Epidemiological Studies of Pulmonary Histoplasmosis in a Farm Family. Am. J. Hyg., *55*: 392, 1952.
25. McVickar, D. L.: Growth of *H. Capsulatum*. J. Path. & Bact., *62*:137, 1951.
26. Mochi, A., and Edwards, P. Q.: Geographic Distribution of Histoplasmosis and Histoplasmin Sensitivity. Bull. World Health Org., *5*:259, 1952.
27. Nilzen, A., and Paldrok, H.: A Laboratory Infection Caused by *H. Capsulatum*. Acta. Dermat. Venereol., *33*:329, 1953.
28. Palmer, C. E.: Non-tuberculous Pulmonary Calcification and Sensitivity to Histoplasmin. Pub. Health Rep., *60*:513, 1945. Geographic Differences in Sensitivity to Histoplasmin among Student Nurses. Pub. Health Rep., *61*: 475, 1946.
29. Parsons, R. J., and Zarafonetis, C. D. J.: Histoplasmosis in Man. Arch. Int. Med., *75*:1, 1945.
30. Payne, N. S., Hennigar, G. R., and Sutton, L. E.: Histoplasmosis Occurrence in Virginia. Virginia M. Month., *79*:430, 1952.
31. Prior, J. A., Saslaw, S., and Cole, C. R.: Experiences with Histoplasmosis. Ann. Int. Med., *40*:221, 1954.
32. Randall, C. C., and McVicker, D. L.: *H. Capsulatum* in Tissue Culture. Proc. Soc. Exper. Biol. & Med., *77*:150, 1951.
33. Riley, W. A., and Watson, C. J.: Darling's Histoplasmosis in the United States. The Possibility of Further Occurrence of Cases. Minnesota Med., *9*:97, 1926.
34. Salvin, S. B.: Complement-Fixation Studies in Experimental Histoplasmosis. J. Lab. & Clin. Med., *43*:259, 1954.

35. Salvin, S. B., and Furculow, M. L.: Precipitins in Human Histoplasmosis. J. Lab. & Clin. Med., *43*:259, 1954.
36. Schwarz, J.: Giant Forms of *H. Capsulatum* in Tissue Explants. Am. J. Clin. Path., *23*:898, 1953.
37. Shull, H. S.: Human Histoplasmosis: A Disease with Protean Manifestations, Often with Digestive System Involvement. Gastroenterology, *25*:582, 1953.
38. Shulz, D. M.: Histoplasmosis of the Central Nervous System. J.A.M.A., *151*: 549, 1953.
39. Shulz, D. M.: Histoplasmosis, a Statistical Morphologic Study. Am. J. Clin. Path., *24*:11, 1954.
40. Smith, C. E.: Coccidioidomycosis. M. Clin. North America, *27*:790, 1943.
41. Suttleff, Hughes, Utrich and Bunket: Active Chronic Pulmonary Histoplasmosis. Arch. Int. Med., 92:571, 1953.
42. Vanbrueseghem, R., Dubois, A., Janosins, P. G., and Burtsaert, P.: Un cas d'histoplasmose africaine; avec une note mycologique sur *Histoplasma dubosii* n. sp. Ann. Soc. belge de méd. trop., 32:569, 1952.
43. Van Pernis, P. A., Benson, M. E., and Holinger, P. H.: Specific Cutaneous Reactions with Histoplasmosis. J.A.M.A., *117*:436, 1941.
44. Zarafonetis, C. J. D., and Lindberg, R. B.: Histoplasmosis of Darling; Observations on Antigenic Properties of Causative Agent; Preliminary Report. Univ. Hosp. Bull. Ann Arbor, *7*:47, 1941.
45. Zeidberg, L. D., Ajello, L., Dillon, A., and Laliah, C.: Isolation of *Histoplasma Capsulatum* from Soil. Am. J. Pub. Health, *42*:930, 1952.
46. Zeidberg, L. D., Dillon, A., and Gass, R. S.: Some Factors in the Epidemiology of Histoplasmin Sensitivity in Williamson County, Tennessee. Am. J. Pub. Health, *41*:80, 1951.

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CANDIDA (MONILIA) INFECTIONS IN CHILDREN

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This discussion is designed to correlate scattered reports of different disease processes caused by microorganisms of the genus *Candida*, to emphasize those occurring in infants and children, and to consider the increasing importance of these infections in current practice.

MYCOLOGY

The genus *Candida* (formerly *Monilia*) comprises yeastlike organisms forming no ascospores and capable of producing pseudomycelium in vitro and in tissues. Five species are medically significant, of which *C. albicans* (including its variant *stellatoidea*) is most commonly associated with human disease. Smears stained with methylene blue and cultures on Sabouraud's agar are sufficient for most diagnostic purposes. The description and identification procedures presented by Conant[7] obviate the need for further discussion here.

OCCURRENCE

Organisms of the *Candida* group are widely distributed. The oral carrier rate in healthy persons has varied from 5 to 35 per cent in different studies.[4, 22, 32] Method of examination accounts for part of the difference; throat swabs were positive in 5 per cent of one group of children, but mouth rinsings were positive in 33 per cent.[22] Species of *Candida* other than *albicans* make up less than 25 per cent of the total. Children show no sex difference in carrier rate, but in adults there is a 2:1 predominance in females,[32] probably a reflection of a high vaginal carrier rate.

Fecal carrier rate usually exceeds the oral rate in the same persons,

with a higher proportion of species other than *C. albicans*. Normal skin carriers are rare,[4] even on skin affected by other disease processes, in the absence of recent contact with diseased persons.

Species of *Candida* (only rarely *C. albicans*) have been isolated from dairy products, fruit and vegetable matter and, uncommonly, from soil.[28] However, culture medium exposed to air, even in a nursery with epidemic thrush, has failed to indicate the presence of *Candida*.[2, 20]

DISEASE TYPES

Disease caused by *Candida* may be classed as *superficial*, in which the microorganism has direct access to the surface of the skin, respiratory tract or gastrointestinal tract, or *systemic*, when further spread, presumably hematogenous, has occurred. Positive cultures, in the former type, may be etiologically significant only when organisms are demonstrated histologically in the affected tissues.

Superficial Infection

Mucous Membranes. Thrush, which is the most familiar form of *Candida* infection, is usually benign and is especially common in the neonatal period. White patches, variable in size and often confluent, appear on the tongue, gingiva and buccal mucosa; underlying inflammation increases as the lesion advances. The infant is generally asymptomatic, and the process usually subsides in a few days or weeks, spontaneously or coincidentally with the local application of gentian violet, Zephiran or hydrogen peroxide. In older children, or in infants otherwise in poor health, the infection may become chronic, with the usual symptoms of stomatitis; treatment is generally unsatisfactory (Fig. 21).

American writers have rarely mentioned extension of the oral disease into the esophagus and intestinal tract, yet three British and Australian reports[11, 18, 24] describe forty-eight infant cases showing thrushlike lesions of the esophagus, particularly the lower third. The patients had refusal to feed or swallow, dribbling regurgitation early in feeding, or early postprandial vomiting, often with changed blood. Death was preceded by "toxemia" and was usually associated with aspiration pneumonia. Occasionally thrush was absent, and usually there was no other significant primary disease. Hematogenous spread to the kidney, esophageal perforation with *Candida* empyema, or associated *Candida* mastoiditis occurred in six instances.

Pathologically, *Candida* is the primary invader: hyphae grow into the squamous epithelium, eventually penetrating the basement membrane and causing underlying congestion and leukocytic infiltration (Fig. 22). This duplicates the histologic picture of thrush lesions in the mouth.

Further penetration of the hyphae, even deep into the muscularis, is associated with ulceration and pseudomembrane formation; hyphae sometimes penetrate veins. Though colonies of bacteria are of course seen in the pseudomembrane, the sequence indicates that they are secondary. Although this disorder has never been emphasized as a clinical entity at this Hospital, at least twelve infants autopsied here after nutritional disturbances have shown this pathologic picture.

Fig. 21. Chronic thrush in a boy aged 10 years, with lesions of tongue and angles of lips.

Lesions of the lower intestinal tract are relatively rare and are usually mild and scattered. There is a single report of two infants having ulcerative lesions as the only evident abnormality at death.[21]

Thrush of the vaginal mucosa, extremely common in adults, usually occurs in children only in association with extensive cutaneous or mucous membrane disease.

Skin. The most superficial skin disorder caused by *Candida* is a rapidly developing and active dermatitis of small infants, usually starting beneath the diaper. Tiny vesicles show surrounding erythema and coalesce to form spreading patches of denuded epithelium with a sharply defined, raised, scaling border. The central areas heal with variable speed. Morphologic variations may resemble ringworm, seborrheic dermatitis or, when more severe, Leiner's disease (*erythroderma desquamativa*). *Candida* is easily demonstrated by direct examination and culture of skin scrapings, and the microorganisms are frequently absent from the mouth and stools. This disorder seems infrequent in this country at present, but was common in the past, and two epidemics in Europe have been described recently.[15, 23] Because the infection is self-limited, local treatment is difficult to evaluate.

The intertriginous and immersion type of ringworm-like *Candida* dermatitis of adults, being largely dependent upon occupation, obesity or poor personal hygiene, is infrequent in children. More common is a persistent though superficial dermatitis, usually starting about the mouth, secondary to persistent oral thrush in infancy or early childhood.

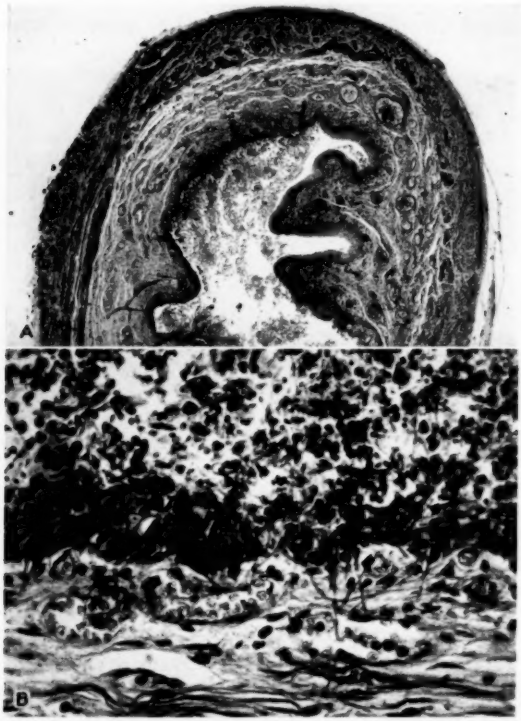

Fig. 22. A, Esophagus of a 2 month female. H. and E. × 15. Note heavy layers of exudate and loss of epithelium over a broad area. *B*, Esophagus of a 17 day premature male. H. and E. × 525. Note blastospores and hyphae in surface debris, partial loss of epithelium, and penetration of hyphae into capillaries of lamina propria. Five hours post mortem.

Papules become scaly, sometimes crusted, and coalesce to form plaques holding more superficial debris than "ringworm" lesions and healing slowly, but without scarring. The process may spread to the scalp, but breaking hairs are absent even though there may be alopecia. Usually the skin about the nails becomes involved, followed by dull red tender paronychia spreading from the nail base. Nails become thickened, scaly and furrowed and may be shed repeatedly (Fig. 23). Though *Candida* may be demonstrated microscopically or by culture, organisms seem

rare, and the fact that the skin lesions in these patients can be reproduced by superficial injections of sterile culture filtrates suggests that sensitization may be important in their pathogenesis. Pathologically, the skin shows nonspecific inflammation.

This disorder is associated remarkably frequently with some major systemic illness (other than disseminated *Candida* infection). Three children presented moderate to severe dwarfing with death from complicating infection after many years.[3, 26] General alopecia sometimes occurs.

In addition to this there is a prominent syndrome of cutaneous *Candida* infection plus hypoparathyroidism or adrenal insufficiency, or

Fig. 23. Chronic paronychia and onychia in a boy aged 6 years (same patient as in Figure 21).

both. Eleven cases have been reported to date,[6, 25, 31] and a review including three more is in preparation.[9] The *Candida* infection generally antecedes the endocrine manifestations by several years, and although the patient usually dies of infection, disseminated *Candida* infection has been present only once. Although it has been impossible thus far to clarify the pathogenetic relationship between the chronic skin infection and the endocrine disorders, the presence of the former should be a strong warning to look for the latter.

A deeper and even more persistent *Candida* infection of the skin is at first similar to the foregoing, but shows many crusts and piling up of hyperkeratoses, with the eventual development of cutaneous horns several centimeters long. The face and scalp are especially affected. When lesions chance to heal, pitted scars remain; inflammation and tissue destruction extend deep into the corium instead of being confined to the epidermis as in the previous form. A further difference is the

presence of large numbers of *Candida* in the lesions. Keratolytics, fungistatic dyes, roentgen irradiation and systemic iodides have been inadequate therapy. Of thirteen cases reported, four patients adequately followed up died of pulmonary infection (non-*Candida*) before age twenty.[14] The disease has not been reported in adults.

As in other cutaneous fungus infections, papular or erythematous id reactions have been noted frequently. As suggested earlier, they may account for a large part of the cutaneous reaction to the infection; they have been reported in an infant as young as five weeks.[15]

Chronic onychia and paronychia may occur as an isolated disease. Fifty-one days is the youngest age reported, in a premature infant.

Respiratory Tract. Candida can undoubtedly cause a variety of lower respiratory tract diseases in adults (more than half the clinical literature on *Candida* infection is devoted to this field), but it is doubtful whether there is a single unequivocal case report of such disease in a child.* Adams' report of eight infants with thrush and pneumonia,[1] quoted in several pediatric texts, lacks proof of *Candida* infection of the lungs, the single autopsy showing only staphylococcal pneumonia. That author's conclusion about synergism between *C. albicans* and *M. pyogenes* var. *aureus* is entirely speculative. Kostmann recorded a young infant with subacute pneumonia and *Candida* in the mouth and stools who responded only to iodide therapy;[16] by comparison with adult experience he presumed this to be *Candida* pneumonitis. Recently Skogrand has reported the first case, in a year old boy dying of acute pneumonia, in which *Candida* was demonstrated in the lung by culture and section.[29]

Several infants in the Children's Hospital who had extensive thrush associated with severe tracheobronchitis and pneumonitis recovered on supportive therapy alone, but in twenty-eight years of autopsy records in this same Hospital the only *Candida* found in the lung (in the absence of disseminated hematogenous infection) have been fragments of mycelium aspirated from thrush or esophagitis. If *Candida* produces significant pulmonary disease in infants and children, pathologic proof is curiously lacking.

Other Sites. Candida mastoiditis associated with esophagitis has already been mentioned. A mild but persistent membranous tonsillitis has been reported, as have infections of the nasolacrimal duct, conjunctiva and cornea. There are several cases of osteomyelitis due to *Candida* alone, as well as subcutaneous abscesses at injection or operation sites. Such infections are rare, but emphasize the importance of examining carefully for fungi a persistent lesion of any sort.

* The eleven year old girl in a family dust-borne infection reported by Hamil is an exception.[13]

Systemic Infection

Systemic infections, often without a manifest superficial source of infection, have been reported occasionally in the past and with increasing frequency in the last few years.

Endocarditis. Most cases of *Candida* endocarditis have been in adults, particularly in heroin addicts using unsterile materials. A seven month infant recently reported ran a sixty-six day course;[17] it was suggested that

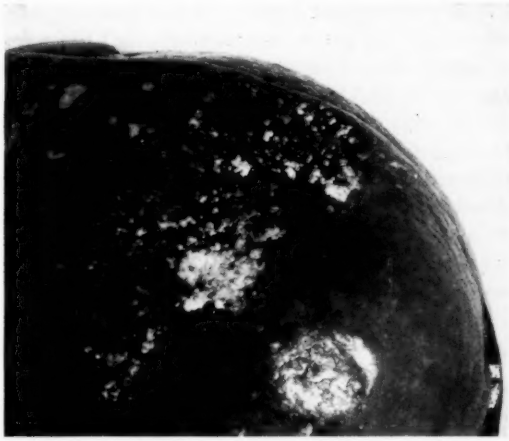

Fig. 24. Mycelial plaques on the surface of the spleen of a 6 year old leukemic boy dying of disseminated *Candida* infection.

a bronchoscopy performed for atelectasis a few days before the onset of clinical thrush might have caused infection through trauma.

The clinical picture does not differ from that of bacterial endocarditis, and it is clear that a blood culture positive for *Candida* must not be unequivocally assumed to be contaminated.

Meningitis. Of the few cases of *Candida* meningitis reported, there has been only one in an infant, aged twenty-two months.[30] Diagnosis was made during life and confirmed at autopsy; thrush was present. An adult patient recovered on sulfonamide and iodide therapy, relapsed, then recovered completely on streptomycin and iodides. The significance of this therapy is uncertain.

Diagnosis is made by culture and direct examination of the cerebrospinal fluid; as in the case of *Cryptococcus* meningitis, the yeastlike cells are easily confused with erythrocytes or lymphocytes.

Urinary Tract. Infrequent but persistent cystitis and pyelitis have been seen in adults, but must be rare in children. An extensive infection in a seven month infant is reported. The emphasis, again, is not upon the

character of the disease, but upon the fact that a positive urine culture must not be assumed to be contaminated.

Disseminated Infection. Until the last few years reports of disseminated *Candida* infection were limited to a few cases in drug addicts, but recently numerous cases have appeared in patients of all ages, with lesions scattered throughout the body (Fig. 24). These lesions vary from small foci of yeastlike cells with little cellular reaction, through acute abscesses with leukocyte accumulation (largely mononuclear) and more or less necrosis, to chronic granulomas with few demonstrable organisms. Thrombosis from growth within veins occurs, and there may be large abscesses and septic infarcts. Every tissue may be involved in an extensive infection. Often superficial lesions of the mouth or esophagus indicate a possible portal of entry, but the one common factor has been intensive antibiotic therapy prior to and during development of the disease. Four cases in infants with nutritional disturbances were recently reported,[10] and at this Hospital in the past nine years there have been over twenty cases in acute leukemias and three in children capable of normal leukocyte responses. Antemortem diagnosis is infrequent and uncertain and is based upon suspicion plus an occasionally positive blood culture.

EPIDEMIOLOGY

Thrush infection of newborn infants is clearly related to maternal vaginal *Candida* infection, which is present in up to 40 per cent of prepartum mothers. Maternal organisms will cause thrush when introduced experimentally into the mouths of newborn infants, and in one study the frequency of thrush in infants with infected mothers was thirty-five times that in the general nursery population.[33] Though this was contradicted by one large study,[20] the attack rate of 6 to 7 per cent in the general nursery population was so high as to obscure any statistical difference between the offspring of infected and of normal mothers. In the latter series, hand contamination was common among infants and attendants, and *Candida* was cultured from rubber nipples and pooled breast milk, indicating other effective modes of spread. Cultures taken daily from newborn infants have become positive in an average of four days after birth, with thrush developing regularly in the next four days. Onset is earlier if the mother is infected.

Beyond the neonatal period, disease caused by *Candida* is sporadic and is apparently dependent upon undefined variations in the environment offered by the host. As noted before, the organism is readily available, especially through personal contact.

IMMUNOLOGY

Many persons develop circulating agglutinins or a cutaneous reaction to extracts of the organisms, or both, indicating merely previous experi-

ence with *Candida* or any of several other genera of yeasts. The incidence of antibodies and skin reactivity increases with age and has reached as high as 50 to 60 per cent in adults. Though the highest titers are usually found in patients with obvious cutaneous infection, the tests are of little diagnostic value.[5, 19]

TREATMENT

Clearly, there is no really effective treatment for *Candida* infections. Gentian violet and the various antiseptics which appear effective in the self-limited oral and cutaneous infections of small infants usually fail in the more persistent infections of older persons. Other agents, such as Castellani's paint (carbol-fuchsin and resorcinol) and various preparations of propionic, caprylic and undecylenic acid, have been used successfully by some and without effect by others. Systemic iodide therapy to the limit of tolerance is routine in adult pulmonary disease;[7] that this may be more than an expectorant effect is suggested by its occasional success in infections elsewhere in the body. However, failures with this agent are common. Roentgen irradiation has occasionally been useful in chronic skin lesions. Stilbamidine, effective against other fungi, is inactive. The various antifungal antibiotics are still in the "may-show-promise" stage, being either too weak or too toxic in vivo.

Concrete recommendations, therefore, are few. Efforts to keep the mucous membranes clean and the skin dry are useful, as are keratolytics on crusted lesions. One cannot escape the conclusion that beyond the neonatal period *Candida* infection becomes established largely in sites or in persons with some underlying abnormality (e.g., occupational dermatoses or complications of diabetes in adults); hence whenever such abnormality can be recognized and understood, treatment should be focused primarily upon it. Unfortunately, as in the aforementioned endocrine disorders, the fundamental host abnormality usually remains obscure.

RELATIONSHIP TO ANTIBIOTIC THERAPY

Coincident with the widespread use of antibiotics there has been an undoubted increase in major *Candida* infections, as evidenced by the large number of recently published reports and by the experience at the Children's Hospital. Between 1927 and 1944 *Candida* infections were limited to superficial sites, whereas since 1945 this Hospital has had about two dozen instances of systemic dissemination.[8] The latter patients had all been treated heavily with antibiotics for another illness.

Experimentally, there is no conclusive evidence in vitro of enhanced *Candida* growth in the presence of any purified antibiotic. In infected chick embryos penicillin treatment has given definitely accelerated

mortality,[12] and the tetracyclines have proved useful in establishing *Candida* infections in mice.[27] Clinically, patients receiving penicillin intermittently over long periods have been noted to acquire active thrush while on penicillin therapy, with subsidence during the intervals.

No definite conclusions are yet possible, and a detailed discussion of the problem is not in order here. However, the practice of avoiding antibiotic therapy in the presence of *Candida* infection, whenever possible, must generally be considered sound until disproved.

As a final note, *laboratory criteria of pathogenicity* (i.e., fermentation reactions, chlamydospore formation, and infectivity for rabbits) *are wholly irrelevant in terms of human disease.* Although *C. albicans* is clearly the commonest species in human infections (and is the only species regularly termed "pathogenic"), deaths have occurred in this Hospital from disseminated disease caused by *C. tropicalis* and *C. pseudotropicalis*, and localized disease is caused by *C. krusei* and *C. parapsilosis* as well. Any species is potentially pathogenic, and a diagnostic screening method which rejects "nonpathogenic" species is a delusion.

REFERENCES

1. Adams, J. M.: A Revaluation of the Pneumonias of Infancy. J. Pediat., *25*:369, 1944.
2. Anderson, N. A., Sage, D. N., and Spaulding, E. H.: Oral Moniliasis in Newborn Infants. Am. J. Dis. Child., *67*:450, 1944.
3. Baird, K. H.: Unusual Syndrome Associated with *Candida Albicans* Infection. Pediatrics, *4*:730, 1949.
4. Benham, R. W., and Hopkins, A. M.: Yeast-Like Fungi Found on the Skin and in the Intestines of Normal Subjects. Arch. Dermat. & Syph., *28*:532, 1933.
5. Carpenter, A., Hadden, J. C., and Spino, P. D.: Incidence of Skin-Test Reactions among Children in the Pittsburgh District. III. Moniliin. Am. J. Dis. Child., *83*:603, 1952.
6. Collins-Williams, C.: Idiopathic Hypoparathyroidism with Papilledema in a Boy Six Years of Age; Report of a Case Associated with Moniliasis and the Celiac Syndrome and a Brief Review of the Literature. Pediatrics, *5*:998, 1950.
7. Conant, N. F.: Manual of Clinical Mycology. 2d ed. Philadelphia, W. B. Saunders Company, 1954.
8. Craig, J. M., and Farber, S.: Development of Disseminated Visceral Mycosis during Treatment of Acute Leukemia. Am. J. Path., *29*:601, 1953.
9. Craig, J. M., Schiff, L. H., and Boone, J. E.: Chronic Moniliasis Associated with Addison's Disease. To be published.
10. Duhig, J. V., and Mead, M.: Systemic Mycosis Due to *Monilia Albicans*. M. J. Australia, *1*:179, 1951.
11. Ebbs, J. H.: Oesophagitis in Infancy. Arch. Dis. Childhood, *13*:211, 1938.
12. Foley, G. E., and Winter, W. D.: Increased Mortality Following Penicillin Therapy of Chick Embryos Infected with *Candida Albicans* var. *Stellatoidea*. J. Infect. Dis., *85*:268, 1949.
13. Hamil, B. M.: Bronchopulmonary Mycosis. Am. J. Dis. Child., *79*:233, 1950.
14. Hauser, F. V., and Rothman, S.: Monilial Granuloma. Arch. Dermat. & Syph., *61*:297, 1950.
15. Holzel, A.: Skin Thrush in Early Infancy. Arch. Dis. Childhood, *28*:412, 1953.
16. Kostmann, R.: Moniliasis of the Respiratory Tract. Acta paediat., *32*:520, 1945.

17. Kunstadter, R. H., MacLean, H., and Greengard, J.: Mycotic Endocarditis Due to *Candida Albicans*. J.A.M.A., *149*:829, 1952.
18. Lederer, H., and Todd, R. M.: Thrush in Infancy. Arch. Dis. Childhood, *24*: 200, 1949.
19. Lewis, G. M., Hopper, M. E., and Montgomery, R. M.: Infections of the Skin Due to *Monilia Albicans*. New York State M. J., 37:878, 1937.
20. Ludlum, G. B., and Henderson, J. L.: Neonatal Thrush in a Maternity Hospital. Lancet, *1*:64, 1942.
21. MacGregor, A. R., and Henderson, J. L.: Intestinal Thrush. Arch. Dis. Childhood, *18*:186, 1943.
22. Marples, M. J., and DiMenna, M. E.: Incidence of *Candida Albicans* in Dunedin, New Zealand. J. Path. & Bact., *64*:497, 1952.
23. Mayer, J. B., Götz, H., and Seitz, G.: Eine Epidemie von Soormykose der Körperhaut unter dem Bilde der Erythrodermia Desquamativa (Leiner). Annales Paediatrici, *177*:213, 1951.
24. Reye, D.: Oesophagitis of Infants. M. J. Australia, 2:673, 1941.
25. Salvesen, H. A., and Boe, J.: Idiopathic Hypoparathyroidism. Acta Endocrinologica, *14*:214, 1953.
26. Schultz, F. W.: Systemic Thrush in Childhood. J.A.M.A., *105*:650, 1935.
27. Seligmann, E.: Virulence Enhancement of *Candida Albicans* by Antibiotics and Cortisone. Proc. Soc. Exper. Biol. & Med., 83:778, 1953.
28. Skinner, C. E.: The Yeast-Like Fungi: *Candida* and *Brettanomyces*. Bact. Rev., *11*:227, 1947.
29. Skogrand, A.: Bronchopulmonal Moniliasis. Nord. Med., *51*:276, 1954.
30. Smith, L. W., and Sano, M. E.: Moniliasis with Meningeal Involvement. J. Infect. Dis., *53*:187, 1933.
31. Sutphin, A., Albright, F., and McCune D. J.: Five Cases (Three in Siblings) of Idiopathic Hypoparathyroidism Associated with Moniliasis. J. Clin. Endocrinol., 3:625, 1943.
32. Todd, R. L.: Studies on Yeast-Like Organisms Isolated from the Mouths and Throats of Normal Persons. Am. J. Hyg., *25*:212, 1937.
33. Woodruff, P. W., and Hesseltine, H. C.: Relationship of Oral Thrush to Vaginal Mycosis and the Incidence of Each. Am. J. Obst. & Gynec., *36*:467, 1938.

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VISCERAL LARVA MIGRANS DUE TO INFECTION* WITH DOG AND CAT ASCARIDS

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Helminths have long been known to produce in human hosts a wide variety of symptoms, which are usually more or less characteristic of the particular infecting parasite. In animal hosts also there are a great number of similar parasitic helminths. What has recently been appreciated, however, is that, though certain parasites are particularly adapted to certain hosts, the relationship is not the "all-or-none" one that had once been envisaged; rather, it can happen by chance that a parasite may penetrate into a host to which it is so imperfectly adapted that it cannot complete its life cycle in that host; yet the larvae are capable of invading the tissues and damaging them to a considerable degree in the course of their fruitless meanderings.

The first clinical syndrome in human beings known to be due to such a foreign parasite is "creeping eruption," also referred to as cutaneous larva migrans; here, skin-penetrating larvae of cat and dog hookworms enter the superficial skin layers sufficiently to produce typical skin lesions. More recently dog and cat ascarids of the genus *Toxocara* have been recognized in the liver, lungs, brain, kidney of children suffering from febrile illnesses associated with eosinophilia.[3, 5, 6, 11] In many other patients eosinophilia and other clinical manifestations, together with a history of eating dirt or of household exposure to dogs and cats, strongly

* *Infestation* is the term which has long been applied to *infection by a large parasite:* modern parasitologists prefer the term *infection,* however, to emphasize the similarities between infection with large parasites, such as nematodes, and infection with small parasites, such as amebae and bacteria, reserving the term *infestation* to denote presence of ectoparasites such as lice on the body or vermin in clothing, stored food, and the like.

suggest visceral larva migrans associated with *Toxocara* infection.[4, 10, 13, 16]

Dog and cat ascarids are, in our experience, the helminths most likely to be associated with the syndrome of visceral larva migrans in New Orleans, and perhaps elsewhere in the United States; other parasites may turn out to be of considerable importance in other parts of the world. Already it is known that even in the United States other helminths may at times be implicated: Muhleisen has reported *Ancylostoma brasiliense* larvae in the sputum of a man who had had severe creeping eruption a few days previously.[12]

Toxocara canis and *T. cati* belong to the family of ascarids and are closely related as regards both morphology and life cycle to the more familiar *Ascaris lumbricoides*. The eggs reach the soil through fecal contamination; there they develop within a matter of weeks to infective-stage larvae which, if ingested by a child, will migrate through the intestinal wall via the vascular system, reach the liver, lungs, brain, eye and other organs. Then, instead of completing the migration back to the intestine as they would in their normal dog or cat host, they either become encysted and are destroyed, or they continue to migrate through the tissues for many months.

These larval parasites are barely visible to the unaided eye, their length being approximately 400 microns. That they can easily be missed even on microscopic study of histologic sections is made clear by the fact that Wilder,[15] in examining 2300 serial sections from a single eye, found an entire nematode larva contained within twelve such sections.

Observations on patients as well as on experimental animals suggest strongly that for the first few days most of the larvae are in the liver; later they move to the lungs, kidneys, brain, spinal cord, muscles, bone marrow, endocrine glands. So far the only organ in which no larva has been found in any animal is the spleen.

PATHOLOGY

The typical pathologic lesion produced by the larva is a granuloma, consisting of eosinophilic leukocytes and "fibrinoid" connective tissue, surrounded by epithelioid cells; the adjacent tissue is heavily infiltrated with eosinophils and in man and monkeys often contains Charcot-Leyden crystals. The granuloma apparently tends to be adjacent to a venule.[5, 6]

The factors which control the extent of the damage produced by Toxocara larvae in the tissues are not yet fully understood. The number of larvae ingested and the frequency of ingestion are probably important. An underlying tendency to hypersensitivity may play a part in the syndrome: Milburn and Ernst[11] point out that histories from five of

fifteen patients which they were able to collect from the literature gave evidence of sensitivity to common allergens.

GEOGRAPHIC DISTRIBUTION

Human infection with Toxocara has a broad geographic distribution. First described in the South, the visceral larva migrans syndrome has now been recognized in many states, in England and in Puerto Rico.

SYMPTOMS AND SIGNS

The disease occurs in children usually between the ages of eighteen months and three or four years. A history of dirt eating and close familial contact with dogs or cats can usually, but not always, be elicited. The symptoms of the disease are variable. In some patients eosinophilia is discovered on the occasion of a blood cell count for some unrelated condition. In other cases medical attention is sought because of intermittent fever, loss of appetite, failure to gain weight, cough, muscle and joint pains, abdominal pain. Many patients suffer from severe impetiginous lesions, specially on the buttocks and legs, others from purpuric skin lesions or nodules rather like erythema nodosum. The one constant finding on physical examination is hepatomegaly, though pneumonitis, abdominal distention and skin rashes are frequent, and splenomegaly has been noted on several occasions. Convulsions and petit mal attacks have been seen.

The probable occurrence of eye damage due to Toxocara migration is noteworthy. The larvae of several species of nematodes have been known to cause choroiditis, iritis, hemorrhages into the vitreous and the retina, cataracts, pseudoglioma, Coats' disease.[7, 15] Recently Wilder,[15] at the Armed Forces Institute of Pathology, carried out a study on the eyes of children (aged three to thirteen years) enucleated because of a clinical diagnosis of retinoblastoma; in twenty-four eyes nematode larvae were found.

Of interest is the matter of cerebral damage. In the series of fifteen patients reviewed by Milburn and Ernst,[11] two suffered from convulsions. One child with the visceral larva migrans syndrome displayed larvae in large numbers in the brain at autopsy;[1] in another a Toxocara larva was a chance finding.* Mice,[14] rats, hamsters, rabbits, guinea pigs and monkeys† infected with Toxocara have all shown larvae in large numbers in the brain. Other types of immature nematodes are known to cause disease of the central nervous system in animals in the Orient, attaining even proportions of "epizootic cerebrospinal nematodiasis."[9]

* P. C. Beaver: Unpublished observations.
† P. C. Beaver and M. H. D. Smith: Unpublished observations.

It is not possible at present to assess the importance of damage to the central nervous system in human beings from Toxocara, but only to point out the likelihood of its occurring more frequently than we have so far realized.

Laboratory data on the patients with Toxocara infection regularly show hyperleukocytosis and eosinophilia varying from 20 to 80 per cent. Hyperglobulinemia is often present to some degree. Liver function tests yield results which are usually within normal limits. Pulmonary infiltration can often be seen in roentgenograms of the chest. The stools show no Toxocara ova, since Toxocara larvae seldom mature in the human intestine.

DIAGNOSIS

The diagnosis of Toxocara infection must be established on clinical grounds, though in severe cases it may be confirmed by demonstrating larvae on liver biopsy. Examination both of stained serial sections and of partially digested fresh tissue may be necessary for such a diagnosis. Preliminary investigations suggest that a skin test may in the future permit accurate recognition and differentiation of infection with Toxocara and Ascaris respectively,* but its value and specificity are not yet established.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis may include, depending on the particular case, pneumonia, miliary tuberculosis, asthma, whooping cough, eosinophilic leukemia, Loeffler's syndrome, familial eosinophilia, retinoblastoma, endophthalmitis. It is well to remember that the presence of Ascaris ova in the stools by no means rules out Toxocara infection, since the child may simultaneously have ingested ova of both nematode species.

PROGNOSIS

The prognosis in this infection is generally good, though in severe cases symptoms may persist intermittently for many months. Two patients in whom the date of infection was accurately known displayed eosinophilia for twelve months.[14] Zuelzer[16] reports eosinophilia persisting for three years. Deaths are known to have occurred in three patients, one a three year old child reported by Zuelzer,[16] another a two year old boy from New Jersey,[5] the third a patient from New Orleans.[2]

* R. C. Jung: Unpublished observations.

TREATMENT

Treatment is at present purely symptomatic. Arsenicals, Hetrazan and cortisone have all so far proved disappointing. Desensitization has been tried, but the results have not yet been confirmed.[7]

PREVENTION

Prevention is best accomplished by protecting small children from contact with cats and dogs, particularly with kittens and puppies, since older animals apparently acquire resistance to Toxocara infection.[8] * When contact between children and animals cannot be avoided, then the pets should be dewormed at frequent intervals (e.g., every four weeks for animals under six months of age, every six to eight weeks for dogs and cats between the ages of six and twenty-four months). Before turning small children loose to play in an outdoor area where pets have recently been confined, the topsoil may be turned under to put the nematode ova out of reach. There is no chemical which can be used to kill the ova in soil.

Elimination of the visceral larva migrans syndrome in children clearly depends upon alerting public health authorities, general practitioners, pediatricians and veterinarians—and, through them, the general public—to the dangers of close association between young children and young household animals.

REFERENCES

1. Beautyman, W., and Woolf, A. L.: An Ascaris Larva in the Brain in Association with Acute Anterior Poliomyelitis, J. Path. & Bact., 63:635, 1951.
2. Beaver, P. C.: Parasitic Diseases of Animals and Their Relation to Public Health. Vet. Med., 49:199, 1954.
3. Beaver, P. C., Snyder, C. H., Carrera, G. M., Dent, J. H., and Lafferty, J. W.: Chronic Eosinophilia Due to Visceral Larva Migrans. Pediatrics, 9:7, 1952.
4. Behrer, M. R.: Hypereosinophilia with Eosinophilic Granuloma of the Liver Associated with Ascaris Infestation. J. Pediat., 38:635, 1951.
5. Brill, R., Churg, J., and Beaver, P. C.: Allergic Granulomatosis Associated with Visceral Larva Migrans. Case Report with Autopsy Findings of Toxocara Infection in a Child. Am. J. Clin. Path., 23:1208, 1953.
6. Dent, J. H., and Carrera, G. M.: Eosinophilia in Childhood Caused by Visceral Larva Migrans. J. Louisiana State Med. Soc., 105:275, 1953.
7. Drouet, P. L., Thomas, C., Cordier, J., and Algan, B.: Origine parasitaire de certaines hemorragies recidivantes du vitré. Bull. et mém. Soc. franc. d'opht., 62:250, 1949.
8. Hinman, E. H., and Baker, D. D.: Helminthological Survey of 1315 Dogs from New Orleans, with Special Reference to Age-Resistance. J. Trop. Med., 39: 101, 1936.
9. Innes, J. R. M., and Shoho, C.: Nematodes, Nervous Disease, and Neurotropic Virus Infection. Brit. M. J., 2:366, 1952.
10. Mercer, R. D., Lund, H. Z., Bloomfield, R. A., and Caldwell, F. E.: Larval

* F. A. Ehrenford: Personal communication.

Ascariasis as a Cause of Chronic Eosinophilia with Visceral Manifestations. Am. J. Dis. Child., *80*:46, 1950.
11. Milburn, C. L., Jr., and Ernst, K. F.: Eosinophilia-Hepatomegaly Syndrome of Infants and Young Children. Pediatrics, *11*:358, 1953.
12. Muhleisen, J. P.: Demonstration of the Pulmonary Migration of the Causative Organism of Creeping Eruption. Ann. Int. Med., *38*:595, 1953.
13. Perlingiero, J. G., and György, P.: Chronic Eosinophilia: Report of a Case with Necrosis of Liver, Pulmonary Infiltrations, Anemia and Ascaris Infestation. Am. J. Dis. Child., *73*:34, 1947.
14. Smith, M. H. D., and Beaver, P. C.: Persistence and Distribution of Toxocara Larvae in the Tissues of Children and Mice. Pediatrics, *12*:491, 1953.
15. Wilder, H. C.: Nematode Endophthalmitis. Tr. Am. Acad. Ophth., *55*:99, 1950.
16. Zuelzer, W. W., and Apt, L.: Disseminated Visceral Lesions Associated with Extreme Eosinophilia. Am. J. Dis. Child., *78*:153, 1949.

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CONGENITAL AND ACQUIRED TOXOPLASMOSIS*

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Toxoplasmosis is a comparative newcomer among the recognized human diseases of infectious etiology. The causative agent, *Toxoplasma gondii*, was first proved to produce illness in man in 1939 when Wolf, Cowen and Paige demonstrated the parasite in cases of congenital encephalomyelitis. The organism had been found in animals in 1908 by Nicolle and Mangeau in North Africa, and in Brazil by Splendore; that it could cause human disease was suspected during the intervening thirty-one years, but proof was not available until Wolf, Cowen and Paige isolated toxoplasma from a patient.

Interest in the human disease developed rapidly, but progress was limited to a large extent because of the limitations imposed by the available techniques. The majority of the recognized cases were of the congenital variety, and it was determined that in this condition there might occur prematurity, stillbirth, hydrocephaly, microcephaly, chorioretinitis, cerebral calcifications, psychomotor retardation and convulsions. Several instances of acquired toxoplasmosis also were reported, and these took the form of either encephalitis or a rickettsial-like illness with a rash.

A number of workers attempted to relate toxoplasma to various clinical problems of undetermined cause, such as acquired chorioretinitis, epilepsy, hydrocephaly, and the like, but definitive answers for these questions were not possible, mainly because of the lack of proper technical procedures. The development of several laboratory methods during the past six to seven years has helped advance our knowledge of human toxoplasmosis markedly, but much still remains to be learned before our understanding of this disease even begins to resemble completeness.

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THE PARASITE

Toxoplasma gondii is an oval or crescentic organism 2 to 4 by 5 to 7 microns which is rather fragile and divides by binary fission. It is probably a protozoon and is identified best with Wright and Giemsa stains. Toxoplasma multiplies only in the presence of living cells and has the unusual characteristic of being able to invade any tissue of mammals and birds except, perhaps, erythrocytes. The parasite is readily spun down in the centrifuge and does not survive freezing.

EPIDEMIOLOGY

Evidence has accumulated that toxoplasma may be found throughout the temperate and tropical zones. The incidence of antibodies (dye test) among "normal" populations appears to vary considerably from place to place, so that such determinations must be interpreted with some caution. For example, we have found significant amounts of antibody to be present in only 4 per cent of Navajo Indians, 17 per cent of residents of Portland, Oregon, 35 per cent of residents of Pittsburgh, and 68 per cent of Tahitians. The demonstration of antibodies in single serum samples has different degrees of importance in each of these populations. Similar differences undoubtedly will be found in other areas as well.

Antibodies are found with equal frequency in both sexes. Evidence for infections exists among many animal species and some birds (especially pigeons). This is especially true of dogs and cats. There is no evidence to suggest, as yet, that infection is transmitted from man to man. We do not know how the infection is acquired by human beings; transmission via insects has been suspected, but remains in the realm of speculation.

PATHOLOGY

Histologic changes may be found in all tissues, but especially in the central nervous system and eyes in the congenital cases and in the lymph nodes and muscles in the acquired cases. Gross and microscopic areas of necrosis may be noted in the lungs, heart, liver, spleen and skeletal muscle. Small and large areas of calcification are seen in the brains in the congenital form and are probably the result of hemorrhages due to destruction of vessel walls. Pseudocysts containing many organisms may be found in the brain and muscles, frequently in the absence of any surrounding area of reaction.

Siim[7] believes that characteristic changes may be noted in biopsied lymph nodes obtained from acquired cases. He describes these as having a normal architecture with hyperplastic lymphatic tissue containing

large pale cells with deep-stained nuclei and many mitotic figures. Scattered throughout, but especially in the follicles, are many macrophages which contain small, deep-stained granules in their cytoplasm. Whether these basophilic granules represent nuclear debris or toxoplasma is not known. Siim has isolated toxoplasma from such a node, and Pinkerton has demonstrated pseudocyst formation in similar specimens.

HUMAN TOXOPLASMOSIS

The disease occurs in two forms: (*a*) congenital, (*b*) acquired. The congenital disease seems always to leave some sort of damage, while the acquired disease may be either symptomatic or inapparent. The latter usually resolves completely, provided the patient recovers.

Congenital Toxoplasmosis

In our experience, congenital toxoplasmosis occurs in the absence of any recognizable disease in the mother, probably as the result of an inapparent infection acquired during pregnancy and accompanied by parasitemia. Thus we have no way of detecting (or suspecting) the infection prior to the birth of the infant. Approximately one quarter of these infants are born prematurely, and the greatest number of deaths occurs in this group.

The infant born with congenital toxoplasmosis may have fever, microcephaly or hydrocephaly, icterus, generalized maculopapular rash, splenomegaly, hepatomegaly, lymphadenopathy, convulsions, chorioretinitis, ocular palsies and microphthalmia. These changes may all be present at birth or appear several days or weeks later, but each patient need not exhibit all these changes. Some infants may appear normal at birth, and then disturbances in head size, convulsions, psychomotor retardation and retinal abnormalities may be noted some weeks or months later. In our experience the chorioretinitis is almost always bilateral, and there is no typical location or degree of involvement.

Acquired Toxoplasmosis

This may present in a variety of ways, but much remains to be learned before the complete spectrum of its clinical expression has been elucidated. In some patients the disease appears in the form of encephalitis with or without a generalized eruption. In others it has appeared with generalized lymphadenopathy, malaise and fever and could be confused with infectious mononucleosis, especially since the peripheral blood contains atypical lymphocytes, except that the Paul-Bunnell reaction is negative. On other occasions, pneumonia, myocarditis and severe myalgias with or without maculopapular rashes have been encountered.

The following represents a typical case of acquired toxoplasmosis as selected from our files.

CASE HISTORY. A white boy, age 3 years, became ill rather abruptly with fever of 105° F. His temperature dropped to 101° F. the next day and remained at a low grade level for some days. Four days after the onset of the acute illness a generalized maculopapular rash appeared and persisted for about a day. At this time generalized lymphadenopathy was noted. The total white blood cell count at the onset was 2500, with 72 per cent lymphocytes, 18 per cent monocytes and 10 per cent polymorphonuclear cells. Three days later this had changed to a total count of 6750, with 61 per cent lymphocytes, 14 per cent atypical lymphocytes and 25 per cent polymorphonuclear cells. The abnormal hematologic picture reverted to normal within about 2 weeks after the onset, and the patient made an uneventful, spontaneous recovery. Toxoplasma serologic studies were consistent with the diagnosis of acquired toxoplasmosis, while the Paul-Bunnell, cold agglutinin and other serologic procedures were all negative. The source of the infection was never determined, nor was infection demonstrated in other members of the family.

LABORATORY STUDIES

Cerebrospinal Fluid

In the active, neonatal infections the cerebrospinal fluid may be xanthochromic and contain an increased amount of protein. The cell count is usually under 200, and some of these leukocytes may be eosinophils. Parasites may be found in such fluid by staining the sediment after centrifugation with either Wright or Giemsa stain. Such sediments may also be injected into mice (which are very susceptible to infection) and the organism subsequently demonstrated in various tissues of animals that become ill or die.

The cerebrospinal fluid of patients with the acquired encephalitic form of toxoplasmosis is indistinguishable from that in any other form of encephalitis except that, on occasion, parasites may be demonstrated.

Hematology

The total white blood cell count is either low, normal or only slightly elevated during the acute illness. It is unusual to find the count to exceed 15,000. The differential smear may be normal or contain an increased percentage of cells of the lymphocytic series. Atypical lymphocytes may be present during the acute phase, and eosinophilia also has been noted during this period.

Roentgenography

Intracerebral calcifications have been seen only in the congenital disease. These may be single or multiple, and their distribution and shape do not follow any characteristic pattern.

Serology

The most widely used test for serum antibodies is the dye test, described by Sabin and Feldman.[5] Such antibodies develop rapidly and appear to reach significant titers within about two weeks after onset of the infection. Titers of 1:16 and above are probably significant, and levels of 1:1024 and higher persist for weeks or months after onset of the infection. The titer gradually diminishes, but such antibodies probably remain detectable for life.

Complement-fixing antibodies appear slowly and disappear more rapidly than do dye test antibodies. It is possible, early in the acute illness, to have a high dye test titer but a negative complement-fixation test. The same, also, may be true some months after the infection has subsided. Consequently, serial serum samples are more informative than are the results of a single examination.

A skin test has been used, but is of no help in a specific case; it may be of some value in broad population studies.

CLINICAL COURSE

The clinical course of toxoplasmosis is unpredictable. Either form of the disease may resolve in a matter of days, weeks or months. In one patient with acute, acquired disease, symptoms persisted until she died almost three months after onset. Some patients with the acquired disease have had prolonged periods of asthenia following the acute illness, possibly as the result of cardiac and skeletal muscle involvement.

MANAGEMENT

Antibiotics are generally ineffective in the treatment of toxoplasmosis. Although sulfonamides seem to suppress experimental infections, they have been disappointing in the treatment of human infections. There is some suggestive experimental evidence that a combination of sulfadiazine and pyrimethamine (Daraprim) is superior to either alone. This combination is worthy of trial in human beings. Since no optimal dosage schedule has been determined, both drugs should be given to tolerance. In infants this would mean 60 mg. per pound per day for the sulfadiazine and 10 mg. per day for the pyrimethamine. Older children probably should receive 15 mg. of the pyrimethamine daily. There is no information on which to base a decision concerning the optimal treatment period, but therapy should be maintained for at least several days after the patient has become asymptomatic.

PROGNOSIS AND SEQUELAE

In our experience with the congenital disease, 27 per cent of those born prematurely have died, in contrast with 12 per cent deaths among those carried to term. Almost all survivors had some degree of disability. Almost all patients had chorioretinitis, and slightly more than half had disturbances in head size, psychomotor retardation or cerebral calcifications.

The mortality rate in the acquired disease is unknown, but fatal cases appear to be unusual; recovery is generally complete.

A mother who has been delivered of one child with congenital toxoplasmosis need not fear similar occurrences in subsequent pregnancies. There is no justification for treating such women "prophylactically" with sulfadiazine during later pregnancies.

SUMMARY

Toxoplasma gondii produces either congenital or acquired infections of human beings. Fortunately, most of the latter cases are inapparent, and the majority of patients with the symptomatic infections seem to recover spontaneously and without sequelae. Almost all infants who survive the congenital disease have residual damage of one sort or another. The source of and the manner in which human infections are acquired are unknown.

REFERENCES

1. Feldman, H. A.: The Clinical Manifestations and Laboratory Diagnosis of Toxoplasmosis. Am. J. Trop. Med. & Hyg., 2:420, 1953.
2. Feldman, H. A., and Sabin, A. B.: Skin Reactions to Toxoplasmic Antigen in People of Different Ages without Known History of Infection. Pediatrics, *4*: 798, 1949.
3. Frenkel, J. K., and Friedlander, S.: Toxoplasmosis. Pathology of Neonatal Disease. Pathogenesis, Diagnosis and Treatment. Public Health Service Publication No. 141. Washington, D.C., U.S. Government Printing Office, 1951.
4. Sabin, A. B.: Complement Fixation Test in Toxoplasmosis and Persistence of the Antibody in Human Beings. Pediatrics, *4*:443, 1949.
5. Sabin, A. B., and Feldman, H. A.: Dyes as Microchemical Indicators of a New Immunity Phenomenon Affecting a Protozoon Parasite (Toxoplasma). Science, *108*:660, 1948.
6. Sabin, A. B., Eichenwald, H., Feldman, H. A., and Jacobs, L.: Present Status of Clinical Manifestations of Toxoplasmosis in Man. Indications and Provisions for Routine Serologic Diagnosis. J.A.M.A., *150*:1063, 1952.
7. Siim, J. C.: Acquired Toxoplasmosis. Report of Seven Cases with Strongly Positive Serologic Reactions. J.A.M.A., *147*:1641, 1951.

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SYMPOSIUM ON LABORATORY TESTS AND SPECIAL PROCEDURES

FOREWORD

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This symposium is designed to focus attention on certain procedures and special problems of interest to those caring for the sick child. It is not intended to be a procedure book or laboratory manual. A perusal of the titles indicates a broad and necessarily spotty coverage; we go from a consideration of functional alterations of organ systems—liver, kidney, pancreas—to a bedside procedure, the measurement of blood pressure. When reference is made to the laboratory, the aim has been to consider procedures that are generally and readily available.

The individual authors have done a splendid job, and as Consulting Editor I take this opportunity to thank each of the contributors for presenting his subject matter in a simple and concise manner.

In current medical practice the laboratory has come to occupy an important and essential place. The preparation of this foreword gives me an opportunity to air some of my views concerning the clinical laboratory. Unfortunately, there is a tendency in some quarters to place data derived from the laboratory above those derived from a careful and complete history and physical examination of the patient. The successful practitioner blends the information with proper perspective, recognizing the limitations of each source of information. Should the laboratory report seem too remote to fit the condition of the patient, the test should be repeated before describing the test as worthless, or before deciding upon a course of therapy which may be costly or present a therapeutic risk. There are many conditions, however, which can be detected only by laboratory investigation, and here the physician is at the mercy of the clinical pathologist. It behooves the clinician, therefore, to have a clear idea of the significance and limitations of each of the methods used in his laboratory if he is to interpret the findings intelligently. The number of tests requested of any hospital laboratory today

are so numerous that the clinical pathologist is often at his wit's end to satisfy the laboratory-minded clinician. The clinician, on the other hand, should justify each procedure he orders. There are times when the assistance of other laboratories is needed, as in the performance of highly specialized and seldom-requested procedures which require special equipment, reagents or "know-how." The clinician, by working hand in hand with the clinical pathologist, will do much to keep the level of medical practice elevated.

LIVER FUNCTION TESTS IN INFANTS AND CHILDREN

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Although special tests for the study of patients with jaundice and liver disease were developed over half a century ago, the ideal test suitable for universal application remains to be devised. Instead, the clinician today is confused by a multiplicity of tests, the results of which may be extremely difficult to interpret. In infants and children these tests are further complicated by the fact that, early in life, the liver is an extremely sensitive organ, easily affected by fever, intercurrent infections or metabolic disorders. These will frequently give rise to falsely positive results. Furthermore, some of the tests useful in children and adults, such as the flocculation tests, are frequently unreliable during infancy.

This paper will discuss practical aspects of liver function tests in infants and children from three viewpoints: (1) selection of tests which are most likely to be helpful in pediatrics; (2) description of these tests and their normal value for infants and children; and (3) clinical application of these tests at different ages. This discussion is based on experience gained at a children's hospital over a seven year period from tests performed on approximately 2500 infants and children. Of these, 10 per cent were healthy normal children who served as controls, 20 per cent were known to have some form of liver disease, and the remainder were patients with various other conditions, but no obvious liver impairment.

CHOICE OF TESTS

Many methods have been devised for determining the functional capacity of the liver. Each of these tests deals with a specific function

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and has its own clinical significance and limitations. The following is a list of some of the tests which are of value in the clinical study of liver function in children, grouped according to the function they attempt to evaluate:

A. Excretion of bile pigments
   1. Serum icterus index, van den Bergh, one-minute and total serum bilirubin
   2. Urine bilirubin and urobilinogen
   3. Stool bilirubin and urobilinogen
B. Formation of serum proteins
   1. Flocculation tests, including thymol turbidity, thymol flocculation, cephalin-cholesterol flocculation, Takata-Ara, colloidal gold, zinc sulfate, cadmium sulfate, and the like
   2. Albumin and globulin determination
   3. Prothrombin time
C. Carbohydrate metabolism—glucose tolerance, levulose tolerance and galactose tolerance
D. Fat metabolism—total cholesterol and cholesterol partition
E. Alkaline phosphatase in serum
F. Serum cholinesterase*
G. Loading tests—bromsulphalein and bilirubin excretion
H. Detoxification tests—hippuric acid test

In reviewing these tests it is obvious that one is faced with the need of selecting a small group which will, on the one hand, give the greatest amount of useful information and, on the other hand, will not require

TABLE 7. *List of Liver Function Tests Found to Be Most Useful in Infants and Children*

MATERIAL	TEST	METHOD	SERUM REQUIRED
Urine	Bilirubin	Free and Free[4]	—
		Klatskin and Bungards[19]	—
	Urobilinogen	Wallace and Diamond[25]	—
Stool	Bilirubin	Harrison[10]	—
		Godfried[7]	
Serum	1-Minute bilirubin	Malloy and Evelyn[21]	0.10 ml.
	Total bilirubin	Ducci and Watson[3]	
		Hsia, Hsia and Gellis[17]	
	Cephalin-cholesterol flocculation	Hanger[8]	0.20 ml.
	Thymol turbidity	Maclagan[20]	0.05 ml.
	Thymol flocculation	Neefe[23]	
	Bromsulphalein	Gaebler[5]	0.10 ml.
		Yudkin and Gellis[27]	
		Total serum required	0.45 ml.

* For a convenient micromethod useful in following the course of illness in patients with liver disease, see the paper by Bleisch and Shwachman: Pediatrics, *13*:426, 1954.

excessive amounts of blood or serum and will be relatively simple to perform in the average laboratory.

The proper choice of tests depends on the general nature of a given pathologic condition. If jaundice is present, the tests chosen should aid in determining whether the jaundice is due to (1) intrinsic, parenchymatous liver damage, (2) extrahepatic obstruction of the bile ducts or (3) a hemolytic process. If jaundice is absent, the tests can help determine (1) whether there is mild or severe involvement of the liver tissue, and (2) whether one is dealing with the early or late stages of the illness. The liver function tests which in our own experience have been found most useful in infants and children are listed in Table 7. All these tests are simple to perform and do not require special equipment. All the tests can be done on 0.45 ml. of serum or approximately 1 ml. of whole blood, which means that, when necessary, they may all be done on capillary blood.

METHODS AND NORMAL VALUES

In the following section the standard methods have been adapted for pediatric use wherever necessary.

Urine Bilirubin and Urobilinogen

Urine Bilirubin.[4, 19] MATERIALS. (1) Special cellulose-asbestos mat. (2) Reagent tablet made up of a special diazonium salt, sulfosalicylic acid and sodium carbonate. (Both these items may be obtained under the name of "Ictotest" by Ames Co., Elkhart, Ind.)

PROCEDURE. Five drops of urine are placed on the center of the special mat. The reagent tablet is placed on the moist mat and flooded with two drops of water which are allowed to run down the side of the tablet. Development of a purple or blue color in the area surrounding the tablet within thirty seconds denotes the presence of bilirubin in the urine.

NORMAL VALUES. The test should be negative for all ages with the exception of the first week of life, when it may become weakly positive in association with physiologic jaundice.

Urine Urobilinogen.[25] REAGENT. Ehrlich's aldehyde reagent, prepared by dissolving 2 gm. of p-dimethylaminobenzaldehyde in 100 cc. of 20 per cent hydrochloric acid.

PROCEDURE. Add 1 cc. of Ehrlich's aldehyde reagent to 10 cc. of fresh urine. The appearance of a pink to cherry red color after five minutes at room temperature is a positive reaction. For a rough estimate of the urobilinogen concentration, the test is repeated with various dilutions of urine (1:10, 1:20 and so on) until a negative reaction is obtained.

NORMAL VALUES. A trace to 1:40 dilution is normal at all ages except for a slight increase during the first week of life, when physiologic jaundice is present.

Interpretation. The incipient and recovery stages of parenchymatous jaundice, such as viral hepatitis, are characterized by marked increases of urine bilirubin and urobilinogen, while the advanced stage of the disease is marked by large amounts of bilirubin, but only small amounts of urobilinogen in the urine. In uncomplicated obstructive jaundice, such

as biliary atresia, there is pronounced urinary excretion of bilirubin, but almost no urobilinogen. When the obstruction is only partial, such as with a choledochal cyst, bilirubin and varying amounts of urobilinogen are found.

Stool Bilirubin

REAGENTS. (1) Fouchet's reagent, prepared by adding 25 gm. of trichloroacetic acid, 10 cc. of 10 per cent ferric chloride to 100 cc. of distilled water; (2) 10 per cent barium chloride solution.

PROCEDURE. Approximately 1 gm. of stool is dissolved in 15 cc. of distilled water, mixed, and separated. Ten cubic centimeters of the supernatant fluid are added to 5 cc. of 10 per cent barium chloride solution and mixed and filtered. After the fluid has passed through the moist filter paper and one or two drops of Fouchet's reagent are added, a green or blue color indicates that bilirubin is present in the stools.

NORMAL VALUES. The test should be positive in infants and children of all ages.

Interpretation. The absence of bile in the stools on several different occasions indicates jaundice due to obstruction. When the obstruction is partial, bile will appear intermittently in the stools.

When jaundice is due to a hemolytic process, the stool urobilinogen will frequently be increased. This can be detected by quantitative methods[22] which are moderately complicated and not considered a routine determination for the average laboratory.

Serum Bilirubin

One-Minute and Total Serum Bilirubin.[3, 17, 21] REAGENTS. (1) Solution A, prepared by adding 1.0 gm. of sulfanilic acid to a liter of water containing 15 cc. of concentrated hydrochloric acid; (2) Solution B, 0.5 per cent sodium nitrate; (3) diazo reagent, prepared fresh each day by adding 0.3 cc. of solution B to 10 cc. of solution A; (4) diazo blank, prepared by adding 15 cc. of concentrated hydrochloric acid to a liter of water; (5) absolute methyl alcohol.

PROCEDURE. One-fifth cubic centimeter of serum* is added to 9.8 cc. of distilled water in Evelyn tube A. Five cubic centimeters of this solution are transferred to Evelyn tube B. One cubic centimeter of diazo blank is added to tube A and placed in the Evelyn photoelectric colorimeter, and, using a 540-millimicron filter, the galvanometer reading is adjusted to 100. One cubic centimeter of diazo reagent is added to tube B, and a reading is taken at the end of exactly one minute. The results are calculated as follows:

$$\text{One-minute bilirubin (mg. per 100 ml.)} = \frac{2 - \log g}{7} \times 250$$

* Collection of blood by capillary method: After the heel of the infant has been punctured, using the tip of a number 11 Bard-Parker blade, blood is collected in chemically clean capillary tubes (8 cm. in length and 0.25 cm. in internal diameter). Better flow of blood can be obtained by soaking the infant's foot in a pan of warm water or rubbing the foot vigorously with an alcohol sponge prior to heel puncture. The specimen in the tubes is capped with rubber caps (manufactured by West Co., Phoenixville, Pa., catalogue number V3-68MS orange) and centrifuged at 1500 revolutions per minute for fifteen minutes. The serum is separated from the cells by filing and breaking at the serum-cell boundary.

In most instances one full tube will yield 0.1 cc. or more of serum, and five tubes should be more than sufficient for all the liver function tests outlined.

Six cubic centimeters of methyl alcohol are then added to each tube, and the results are read again at the end of thirty minutes. They are calculated as follows:

$$\text{Total serum bilirubin (mg. per 100 ml.)} = \frac{2 - \log g}{3.5} \times 250$$

NORMAL VALUES. The one-minute serum bilirubin varies between 0 and 0.2 mg. per 100 ml. both in the cord blood and at all ages thereafter.

The total serum bilirubin tends to rise during the first week of life in association with physiologic jaundice. In full term infants the average level is 2.1 mg. at birth, and rises to 5.7, 6.8, 6.2 and 4.7 mg. per 100 ml. on subsequent days, before returning to a level of 0.8 mg. or less by the end of the first week.[14] The level tends to become higher and remain higher longer in premature infants and will frequently reach 20 mg. per 100 ml. before falling. After the first two weeks the level varies between 0 and 0.8 mg. at all ages.

Interpretation. As a general rule, elevation of total bilirubin without corresponding elevation of one-minute bilirubin indicates jaundice due to hemolysis. Elevation of both one-minute and total bilirubin indicates jaundice due to either parenchymatous liver damage or extrahepatic obstruction. These cannot be distinguished on the basis of bilirubin values alone, but require additional liver function tests.

Flocculation Tests

Cephalin-Cholesterol Flocculation.[8] REAGENTS. (1) Cephalin-cholesterol emulsion, prepared by adding 1 cc. of cephalin-cholesterol antigen (manufactured by Difco Co., Detroit) to 4 cc. of ether (Squibbs anesthesia). One cubic centimeter of this stock ether solution is added to 35 cc. of distilled water warmed to 65° to 70° C. slowly and with stirring. This is then heated slowly to boiling, and the mixture is allowed to simmer until the final volume is reduced to 30 cc. (2) 0.85 per cent saline.

PROCEDURE. One-fifth cubic centimeter of fresh serum is added to 1 cc. of emulsion and 4 cc. of normal saline in a centrifuge tube. This is shaken thoroughly, stoppered, and kept in total darkness at room temperature for twenty-four hours. The tube is then graded 0 to 4+. A 4+ reaction indicates a complete precipitation leaving the supernatant water clear. Gradients between 0 and 4+ are graded 1+, 2+ and 3+.

NORMAL VALUES. Values of 0 to 2+ at the end of twenty-four hours are normal for all ages except in newborn infants, in whom several authors have[2, 24] have reported slight elevations during the first forty-eight hours of life.

Thymol Turbidity.[20] REAGENT. Thymol buffer, prepared by adding 1.38 gm. of barbital, 1.03 gm. of sodium barbital, and 3 gm. of thymol to 500 cc. of water. This is heated to boiling point, cooled, seeded with a small amount of powdered thymol crystals, and filtered after standing overnight.

PROCEDURE. One-twentieth cubic centimeter of serum is added to 3 cc. of buffer for thirty minutes and compared against Kingsbury-Clarke protein standards (manufactured by Hartman-Leddon Co., Philadelphia). If the turbidity should exceed 100 units, the sample can be diluted with 3 cc. of buffer and the readings doubled.

NORMAL VALUES. Values of 0 to 4 units are normal for children of all ages. Desmond[1] has shown that the values tend to be lower in very young infants.

Thymol Flocculation.[23] PROCEDURE. The thymol turbidity tubes prepared as before are kept at room temperature for eighteen hours and read again. The tubes are graded from 0 to 4+ as with the cephalin-cholesterol flocculation test.

NORMAL VALUES. Values of 0 to 1+ at the end of eighteen hours are normal for infants and children of all ages.

Interpretation. The flocculation tests reflect changes in the serum

proteins and generally indicate parenchymatous liver damage. They should be interpreted as a group. They are considered positive if two or more tests are positive or if one test remains consistently positive on several occasions. They are considered negative when all the tests are negative or if one test is positive on one occasion and subsequently becomes negative.

The flocculation tests are useful in following the progress of disease in any given patient. The cephalin-cholesterol flocculation test becomes positive early and the thymol flocculation test remains positive late, long after all clinical symptoms have disappeared.

Unfortunately the flocculation tests are of limited value in infants under six months of age, since they may not become positive even in the face of gross liver damage.[2, 6, 9, 12, 26] The exact reason for this is not clearly understood, but is thought to be related to both the quality and quantity of gamma globulin in the serum of young infants.

Bromsulphalein Excretion Test[5, 27]

REAGENT. Tenth-normal sodium hydroxide.

PROCEDURE. Five milligrams of bromsulphalein per kilogram of body weight are injected into the scalp vein, and a blood sample is taken exactly forty-five minutes afterwards. The bromsulphalein retained is determined by adding 0.1 cc. of serum to 0.6 cc. of tenth-normal sodium hydroxide, and 0.5 cc. of water in an Evelyn microcell (manufactured by Rubicon Co., Philadelphia). The specimen is read against a water blank in the microcell attachment of the Evelyn photoelectric colorimeter, using the 620- and 565-millimicron filters. The milligrams of bromsulphalein retained can be calculated as follows:

$$\text{BSP (Mg./100 ml. serum)} = \frac{L_{565} - 1.3\,L_{620}}{0.095}$$

NORMAL VALUES. Any retention of bromsulphalein in excess of 10 per cent at the end of forty-five minutes is pathologic except in very young infants during the first week of life, when retention of 20 to 30 per cent is not unusual.[27]

Interpretation. Retention of bromsulphalein generally indicates marked damage to the liver parenchyma. It is of special value in following the progress of chronic liver disease, such as hepatitis or cirrhosis, in the absence of jaundice.

CLINICAL APPLICATION

In the Newborn Infant (Birth to 7 Days)

The principal conditions that alter liver function tests during the newborn period are listed in Table 8. Serum bilirubin determinations are most helpful in distinguishing physiologic jaundice from pathologic causes in this age group. One can state as a general rule that total serum bilirubin levels in excess of 10 mg. per 100 ml. in full term infants and

TABLE 8. *Liver Function Tests in Newborn Infants and during Early Infancy*

CONDITION	URINE	STOOL	SERUM			
	Bilirubin	Urobilinogen	Bilirubin	Bilirubin		Flocculation Tests	Bromsulphalein Retention
				1-Minute	Total		
Newborn Period (Birth to 7 Days)							
Physiologic jaundice	Trace	Sl. inc.	Normal	Normal	Elevated*	Neg.	Increased
Erythroblastosis fetalis:							
Due RH	Pos.	Increased	Increased	Normal	Markedly elev.*	Neg.	—
Due ABO	Pos.	Increased	Increased	Normal	Mod. elev.*	Neg.	—
Sepsis	Pos.	Occ. incr.	Increased	Occ. elev.	Elevated*	Occ. pos.	—
Congenital syphilis	Neg.	Occ. incr.	Occ. incr.	Occ. elev.	Elevated*	Pos.	—
Toxoplasmosis	—	—	—	Normal	Elevated*	—	—
Inclusion cell disease	—	—	—	Normal	Elevated*	—	—
Early Infancy (1 Week to 6 Months)							
Prolonged obstructive jaundice:							
Biliary atresia	Pos.	Absent	Absent	Elevated*	Elevated*	Neg.	Increased
Inspissated bile due to eryth. fetalis	Pos.	Decreased	Abs. or decr.	Markedly elev.*	Markedly elev.*	Neg.	—
Neonatal hepatitis	Pos.	Decreased	Abs. or decr.	Elevated*	Elevated*	Usually neg.	Increased
Galactosemia	Pos.	Normal	Normal	Elevated	Elevated	Pos.*	Increased
Hemolytic anemias	Neg.	Increased	Increased	Normal	Sl. elev.*	Neg.	Normal
Prolonged physiologic jaundice	Neg.	Nor. or inc.	Decreased	Elevated*	Elevated*	Neg.	Increased

* Denotes most useful tests.

20 mg. in premature infants[14] during the first week of life are abnormal and suggest a disease process.

Erythroblastosis Fetalis. This is by far the most common and also the most important cause for abnormal jaundice during this newborn period. In this condition clinical jaundice is rarely present at birth, but appears during the first few hours of life and is invariably present by twenty-four hours of age. Measurements of total serum bilirubin will reveal a level of about 5 mg. per 100 ml. in the cord blood, and this may rise to 20 to 30 mg. by twenty-four hours of age, and to 30 to 40 mg. by forty-eight hours of age. Exchange transfusions will markedly lower these levels, owing to a combination of removal of about 85 per cent of the baby's circulating red cells, which are the important source of bilirubin, and removal of the circulating bilirubin itself. Measurements of serum bilirubin levels are most helpful as a guide for determining when exchange transfusions should be performed. In a study on 229 infants with erythroblastosis we[15] found that in the cases in which the range was 0 to 5 mg. per 100 ml. there was no kernicterus, in those in which the range was 6 to 15 mg. there was a 3 per cent incidence of kernicterus, all in infants in whom the only determination was obtained in the first two hours of life. The levels undoubtedly rose much higher in the next forty-eight hours. Kernicterus occurred in 18 per cent of the infants in whom serum bilirubin was recorded at a level between 16 and 30 mg. per 100 ml. and in 50 per cent of those in whom levels above 30 mg. were obtained. Because of these data we have in our hospitals attempted to keep the bilirubin levels below 20 mg. in each case, doing multiple exchange transfusions if necessary. We have not encountered a single case of kernicterus in over 400 consecutive cases of erythroblastosis treated in this way.

Serum bilirubin levels have also been helpful in establishing the diagnosis of erythroblastosis due to ABO incompatibility.[13] In this condition the Coombs test is usually negative and the maternal anti-A or anti-B titer is highly variable. The total serum bilirubin level, however, has invariably been elevated above physiologic limits. Because of this we believe that in the presence of a major blood group incompatibility between the infant and mother with a negative Coombs test, clinical jaundice in the first twenty-four hours, and a serum bilirubin level of more than 10 mg. per 100 ml. at twenty-four hours or less, one is usually justified in making the diagnosis of erythroblastosis due to ABO incompatability and initiate therapy.

Other Conditions. Jaundice during the newborn period can also be caused by sepsis, congenital syphilis, toxoplasmosis and inclusion cell disease. These may be differentiated from physiologic jaundice and erythroblastosis by specific tests, but jaundice is usually a prominent clinical feature. In sepsis and syphilis the excessive bilirubinemia may

cause inspissation of bile and elevations of both the one-minute and total serum bilirubin. The flocculation tests may also become positive, especially in syphilis. This is believed to be due to marked alterations in the serum proteins and is not necessarily indicative of extensive liver damage.

In Early Infancy (1 Week to 6 Months)

Jaundice continues to be the predominant feature in all infants with hepatic dysfunction up to six months of age, as shown in Table 8. However, this is accompanied by more definitive changes in the urine and stools.

Obstructive Jaundice. Prolonged obstructive jaundice in infancy[12] makes up the largest group of cases at this age. These include those with biliary atresia, inspissated bile due to erythroblastosis, and neonatal hepatitis. All infants with these three conditions show the following changes: (1) increased bilirubin in the urine, (2) decreased or absent urobilinogen in the urine, (3) absent or markedly diminished bilirubin in the stools, (4) negative flocculation tests, and (5) elevation of both one-minute and total serum bilirubin. The last differentiates this group from those with physiologic jaundice, erythroblastosis, hemolytic anemias and sepsis, all of which show elevation of total bilirubin without elevation of the one-minute bilirubin.

The serum bilirubin level is the most useful test in differentiating among the three groups. A high initial bilirubin level of 10 to 30 mg. per 100 ml. suggests inspissated bile due to erythroblastosis. A low initial bilirubin level of 5 to 10 mg. suggests either biliary atresia or neonatal hepatitis. If the levels are followed over a period of weeks, more definite diagnostic information can be obtained. If the level shows a slow and steady upward trend with no variability, biliary atresia is strongly suggested. If the level shows a rapid downward trend, inspissated bile due to erythroblastosis is suggested. If the level either shows a tendency to fall slowly or is highly variable, neonatal hepatitis is suggested. When there is doubt with regard to the exact diagnosis, surgical exploration should be postponed until repeated examination of serum bilirubin has been performed over a period of weeks. It has been found that differentiating between biliary atresia and incomplete obstruction is usually not difficult at the end of the waiting period.

Other Conditions. Other conditions that may cause jaundice in this period include galactosemia, various hemolytic anemias and prolonged physiologic jaundice. The ingestion of lactose in infants with galactosemia causes profound disturbances in the liver, elevations of both the one-minute and total serum bilirubin, and also positive flocculation tests and retention of bromsulphalein.[18] Hemolysis can occur at this age as a

result of congenital or acquired hemolytic anemias, Cooley's anemia and sickle cell anemia. All are characterized by the rise of total bilirubin without a corresponding rise of one-minute bilirubin. Except in periods of crisis, the rise seldom exceeds 3 mg. per 100 ml. Finally, jaundice can be caused by prolonged physiologic jaundice. This usually occurs in infants who have been severely ill during early infancy with such conditions as pneumonia, congenital heart disease or renal disease, and the usual physiologic jaundice becomes not only greater, but also more persistent. There is usually elevation of both the one-minute and total serum bilirubin, suggesting inspissation of bile in the liver and bile ducts.

In Older Infants and Children (6 Months to 14 Years)

After the age of six months liver function tests may be interpreted much in the same way as in adults if due allowance is made for the fact that a number of childhood conditions frequently will give falsely positive tests.

Acute Hepatitis. Liver function tests are of the greatest value in the diagnosis and management of patients with acute viral hepatitis. Since it is impossible to differentiate between infectious hepatitis and homologous serum hepatitis either on clinical grounds or by laboratory tests, the two conditions will be considered together. During the very early stages of hepatitis the urine bilirubin test is the first test to become positive. This is followed by changes in the cephalin-cholesterol flocculation test and by retention of bromsulphalein prior to the onset of jaundice. At the height of the disease the urine bilirubin continues to be strongly positive, but the urine urobilinogen and stool bilirubin will become decreased because of the obstruction to the flow of bile caused by liver damage. During this stage all the flocculation tests become markedly positive, the serum bilirubin levels are elevated, and there is increased retention of bromsulphalein. During convalescence the urine bilirubin and urobilinogen return to normal, even before the serum bilirubin. The thymol flocculation test, however, may remain positive long after the disappearance of clinical symptoms.

Although the majority of patients with viral hepatitis recover completely, a small number will continue to show signs of chronic hepatitis for months or years afterwards. These patients have persistently positive flocculation tests and continue to show bromsulphalein retention. When they are fatigued, mild icterus develops and bilirubin appears in the urine. Whether these patients will eventually have cirrhosis of the liver remains to be determined.

Cirrhosis of the Liver. Cirrhosis of the liver shows few changes during the early stages.[16] Except for a slight increase in bromsulphalein retention, all the other tests continue to be normal for many months. As

TABLE 9. *Liver Function Tests in Older Children*

CONDITION	URINE	STOOL	SERUM			
	Bilirubin	Urobilinogen	Bilirubin	Bilirubin		Flocculation Tests	Bromsulphalein Retention
				1-Minute	Total		
Acute hepatitis:							
Early	Pos.*	Sl. inc.	Sl. inc.	Normal	Normal	Ceph.—chol. pos.	Moderate
Advanced	Str. Pos.	Decreased	Decreased	Markedly elev.*	Markedly elev.*	All tests strongly pos.*	Marked
Convalescent	Neg.	Normal	Normal	Normal	Normal	Thy. floc. pos.*	Slight
Chronic hepatitis	Usually neg.	Normal	Normal	Usually normal	Usually normal	Pos.	Moderate
Cirrhosis of liver:							
Early	Neg.	Normal	Normal	Normal	Normal	Neg.	Slight
Late	Pos.	Normal	Normal	Elevated	Elevated	Pos.*	Moderate
Infectious mononucleosis	Neg.	Normal	Normal	Usually normal	Usually normal	Pos.*	Normal or mod. inc.
Granulomas and tumors	Neg.	Normal	Normal	Normal	Normal	Neg.	Normal

* Denotes most useful tests.

the disease progresses, some of the flocculation tests become positive, and there may be elevation of both the one-minute and total serum bilirubin.

Granulomas and Tumors. Granulomas and tumors of the liver are similarly difficult to diagnose during the early stages, and the tests become positive only when the disease becomes far advanced. In these conditions the ultimate diagnosis can be established only on the basis of liver biopsy.

Infectious Mononucleosis. In addition to being of value in the study of patients with known liver disease, the flocculation tests are of special value in helping to establish the diagnosis of infectious mononucleosis. In a study of sixty-two children with a presumptive diagnosis of infectious mononucleosis, Hsia and Gellis[11] showed that 84 per cent of the patients with a heterophile titer of 1:56 or higher showed positive flocculation tests. This is probably related to the diffuse hepatitis which is characteristic of patients with that condition.

FALSELY POSITIVE FLOCCULATION TESTS

Falsely positive flocculation tests are known to occur in association with a number of childhood diseases, where there is little evidence to suggest hepatic dysfunction. Table 10 lists the conditions which in our

TABLE 10. *Conditions Which May Give Falsely Positive Flocculation Tests in Infants and Children*

CONDITION	PERCENTAGE OF CASES WITH POSITIVE FLOCCULATION TESTS
Lupus erythematosus	80
Cooley's anemia	55
Acquired hemolytic anemia	47
Iron deficiency anemia	33
Leukemia and other tumors	29
Tuberculosis	29
Rheumatoid arthritis	28
Acute glomerulonephritis	25
Nephrosis	25
Cystic fibrosis of pancreas	12

experience have given falsely positive tests. In any interpretation of liver function tests in children these conditions must be considered and ruled out. If there is serious doubt as to whether the positive flocculation tests are due to liver disease or one of the other childhood conditions, the bromsulphalein test is most helpful in differentiating between the two. Although the exact cause for these positive tests is somewhat obscure, they are believed to reflect the marked alterations in both the quality

and quantity of serum proteins which are characteristic of most of these conditions.

SUMMARY

The most important aspects of liver function tests in infants and children have been reviewed. Tests should be selected with great care so that they will both provide the needed information and at the same time be relatively simple to perform and require only small amounts of blood or serum. Interpretation of these tests should be undertaken with the following considerations in mind: (1) the special forms of jaundice and liver disease that are characteristic of each age group, (2) the failure of the flocculation tests to function in very young infants, and (3) the greater incidence of falsely positive tests because of other childhood conditions. Despite these limitations, liver function tests can be of great value in the diagnosis and management of many childhood diseases.

REFERENCES

1. Desmond, H. D., Zimmerman, H. J., Sweet, L. K., and Thomas, L. J.: Thymol Turbidity Values in the Sera of Newborn and Premature Infants, and of Mothers at Term. Pediatrics, 3:49, 1949.
2. Drake, M. E.: Infectious Hepatitis in Infancy and Childhood. M. Clin. North America, 36:1637, 1952.
3. Ducci, H., and Watson, C. J.: The Quantitative Determination of Serum Bilirubin, with Special Reference to the Prompt Reacting and Chloroform-Soluble Type. J. Lab. & Clin. Med., 40:610, 1952.
4. Free, A. H., and Free, H. M.: A Simple Test for Urine Bilirubin. Gastroenterology, 24:414, 1953.
5. Gaebler, O. H.: Determination of Bromsulphalein in Normal, Turbid, Hemolyzed, or Icteric Serums. Am. J. Clin. Path., 15:452, 1945.
6. Gellis, S. S., Craig, J. M., and Hsia, D. Y.-Y.: Prolonged Obstructive Jaundice in Infancy. IV. Neonatal Hepatitis. Am. J. Dis. Child., 88:285, 1954.
7. Godfried, E. G.: Clinical Tests for Bilirubin in Urine. Biochem. J., 28:2056, 1934.
8. Hanger, F. M.: Serological Differentiation of Obstructive from Hepatogenous Jaundice by Flocculation of Cephalin-Cholesterol Emulsions. J. Clin. Investigation, 18:261, 1939.
9. Harris, R. C.: Liver Function Tests in Infancy. Bull. New York Acad. Med., 28: 721, 1952.
10. Harrison, G. A.: Chemical Methods in Clinical Medicine. London, J. & A. Churchill, Ltd., 1937, p. 18.
11. Hsia, D. Y.-Y., and Gellis, S. S.: Hepatic Dysfunction in Infectious Mononucleosis in Children. Am. J. Dis. Child., 84:175, 1952.
12. Hsia, D. Y.-Y., and Gellis, S. S.: Prolonged Obstructive Jaundice in Infancy. III. Liver Function Tests. Am. J. Dis. Child., 85:13, 1953.
13. Hsia, D. Y.-Y., and Gellis, S. S.: Studies on Erythroblastosis Due to ABO Incompatibility. Pediatrics, 13:503, 1954.
14. Hsia, D. Y.-Y., Allen, F. H., Jr., Diamond, L. K., and Gellis, S. S.: Serum Bilirubin Levels in the Newborn Infant. J. Pediat., 42:277, 1953.

15. Hsia, D. Y.-Y., Allen, F. H., Jr., Gellis, S. S., and Diamond, L. K.: Erythroblastosis Fetalis. VIII. Studies of Serum Bilirubin in Relation to Kernicterus. New England J. Med., *247*:668, 1952.
16. Hsia, D. Y.-Y., Gellis, S. S., and Craig, J. M.: Cirrhosis of the Liver in Infants and Children. To be published.
17. Hsia, D. Y.-Y., Hsia, H. H., and Gellis, S. S.: A Micromethod for Serum Bilirubin. J. Clin. Investigation, *18*:261, 1939.
18. Hsia, D. Y.-Y., Hsia, H. H., Green, S., Kay, M., and Gellis, S. S.: Amino-Aciduria in Galactosemia. Am. J. Dis. Child., *88*:458, 1954.
19. Klatskin, G., and Bungards, L.: An Improved Test for Bilirubin in Urine. New England J. Med., *248*:772, 1953.
20. Maclagan, N. F.: Thymol Turbidity Test: A New Indication of Liver Dysfunction. Nature, London, *154*:670, 1944.
21. Malloy, H. T., and Evelyn, K. A.: The Determination of Bilirubin with the Photoelectric Colorimeter. J. Biol. Chem., *119*:481, 1937.
22. Mills, S. D., and Mason, H. L.: Values for Fecal Urobilinogen in Childhood. Am. J. Dis. Child., *84*:322, 1952.
23. Neefe, J. R.: Results of Hepatic Tests in Chronic Hepatitis without Jaundice. Gastroenterology, *7*:1, 1946.
24. Salmon, G. W., and Richman, E. E.: Liver Function in the Newborn Infant. J. Pediat., *23*:522, 1943.
25. Wallace, G. B., and Diamond, J. S.: Significance of Urobilinogen in the Urine as a Test for Liver Function. Arch. Int. Med., *35*:698, 1935.
26. Weller, S. D. V.: Observations on Some Liver Function Tests in Infancy. Great Ormond St. J., *1*:26, 1951.
27. Yudkin, S., and Gellis, S. S.: Liver Function in Newborn Infants. Arch. Dis. Childhood, *24*:12, 1949.

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KIDNEY FUNCTION TESTS IN INFANTS AND CHILDREN

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A physician treating an infant or child suspected of having kidney disease is confronted with the problem of selecting appropriate methods for estimating kidney function. The recent development by kidney physiologists of refined tests for measuring discrete kidney functions[17] has made the problem of selection of proper tests more difficult for the clinician. There is uncertainty as to whether or not proper clinical evaluation of a patient with known or suspected kidney disease now requires the use of the more refined methods and, if so, whether or not the tests can be performed and interpreted by one not expert in kidney physiology. A main purpose of this paper will be to consider these pertinent questions and to discuss the value and limitations of tests which, in the judgment of the authors, should be used clinically to evaluate kidney function in infants and children. In the final section the use of these tests in the more common diseases of the kidney will be discussed. References to recent descriptions of the methods and procedures used in the Department of Pediatrics at The New York Hospital-Cornell Medical Center and descriptions of some of the procedures used are given in an appendix.

MEASUREMENT OF DISCRETE KIDNEY FUNCTIONS

Among the important discrete functions of the kidney which can be measured reliably by the techniques of the kidney physiologists are the following: effective renal plasma flow, which in the normal adult is about 600 to 650 ml. per minute; rate of glomerular filtration of water, about 120 to 130 ml. per minute; and rates of absorption and excretion by the tubules of various endogenous and exogenous substances.[17] Com-

pared with those in the adult on the basis of surface area, average values for these functions are lower in young infants,[4, 8, 13, 15, 16, 18, 19] although in older infants and in children they are the same.[16, 19]

The methods currently used by kidney physiologists have been and can be used in both normal and sick infants and children.[4, 8, 13, 15, 16, 18, 19] However, it is the opinion of the present authors that the problems involved in technique (catheterization, infusions, multiple blood samples, special laboratory methods) and in interpretation are of sufficient magnitude that, particularly in infants and children, these methods are not suitable for general clinical application. Furthermore, with few exceptions, the results obtained by these methods thus far have not provided specific aid in the clinical evaluation or treatment of patients with diseases involving kidney function.

Even though the methods themselves may not have general clinical application, an understanding of the *principles* of the methods is important for the clinician. For example, several tests of kidney function which are widely used clinically (urea clearance, phenolsulfonphthalein test) actually represent attempts to estimate the discrete functions which the more refined tests measure reliably.

KIDNEY FUNCTION TESTS SUITABLE FOR GENERAL CLINICAL APPLICATION

Clearances of Endogenous Substances

The most accurate of the clinically applicable tests of kidney function are those which measure clearances of endogenous substances. As in the more refined tests in which exogenous substances given by infusion are used, these clearances are based upon the simultaneous determination of the concentration of a substance in blood or plasma, P, and the rate of its excretion, UV (where U equals concentration of the substance in urine and V equals rate of urine flow). The clearance, C, calculated as UV/P, expresses the minimal volume of plasma required to supply the quantity of the substance excreted into the urine in one minute.[17]

Urea Clearance. The clearance of urea has been and is used clinically more frequently than any other clearance test. Although it does not measure glomerular filtration rate, changes in the urea clearance are interpreted as reflecting changes in the rate of glomerular filtration of water. As with any clearance test, it has the advantage over other clinical methods of estimating kidney function that it relates rate of excretion to concentration in blood. It has the additional advantage that the determination of urea in blood and urine is relatively simple and is done in many clinical laboratories. The urea clearance has two main disadvantages: the ratio of the clearance of urea to the clearance of inulin (normally 0.5 to 0.6 at moderate rates of urine flow) and therefore to the rate of glomerular filtration is markedly changed at different

rates of urine flow and in some diseases of the kidney,[6] and, as in any clearance test, the results depend upon accurate collection of timed samples of urine.

On the basis of these limitations, justified criticisms have been made of the validity of the urea clearance as an estimate of kidney function in children with or without kidney disease.[6] However, it is the opinion of the authors that the urea clearance is clinically applicable* and at present is the most useful test for detecting minimal to moderate decreases or for following changes in kidney function.

Creatinine Clearance. The clearance of "true" endogenous creatinine has the important advantage over the urea clearance that, at least in normal infants and children, it approximates the clearance of inulin and therefore measures glomerular filtration rate.[11, 14] However, the clearance of creatinine also has distinct disadvantages: in the presence of reduced kidney function the creatinine clearance is higher than the inulin clearance,[14] and the determination of "true" serum creatinine (as distinguished from creatinine chromogen) is a relatively difficult procedure which is not generally available. Although it is useful in certain clinical investigations, it is the opinion of the authors that the clearance of creatinine is not useful for general application.

Measurement of Rate of Excretion of Injected Substances

The renal excretion of phenolsulfonphthalein (PSP) is used commonly to estimate kidney function by measuring the fraction of a

* Urea clearances can be done in older children in an office or outpatient department with less difficulty than is generally believed. With proper preparation of the patient (see Appendix), two timed specimens of urine voided spontaneously and a single blood sample can usually be obtained in one to two hours. The differences between the values for the two periods may be large under these circumstances, and differences in results from one visit to another must be interpreted accordingly. The magnitude of the difference in results between two consecutive periods done in this way was estimated from an analysis of 123 clearances done over a period of one year (July, 1953, to July, 1954) in the Renal Clinic of New New York Hospital-Cornell Medical Center. Clearance values over 120 per cent of average normal were not included in this analysis, since even large differences between the two periods did not affect the clinical evaluation of the results. In the 123 clearances in which the values were fairly equally distributed between four groups (0 to 30 per cent, thirty-one clearances; 31 to 60 per cent, thirty-three clearances; 61 to 90 per cent, thirty clearances; and 91 to 120 per cent, twenty-nine clearances) the average standard deviation of the difference between the two consecutive periods was 26 per cent. However, in those clearances (seventy-one of 123) in which the difference in rate of urine flow between the two periods was less than 100 per cent, the average standard deviation was 7. These results indicate that when the difference between the two periods in rate of urine flow is small, the results of the clearances are reasonably reliable.

In infants under a year the urea clearance is a less useful test of kidney function and usually is not done clinically. In addition to the technical difficulty of collecting urine by catheterization, interpretation of the results is complicated by the physiologic immaturity of kidney function and the lack of adequate normal values in infants of different ages up to one year.

known dose recovered in the urine in successive periods after injection of the dye. Although the results of this test may roughly parallel changes in renal plasma flow, its value rests solely upon empirical correlation with other clinical data.[17] It has the advantage, especially for urologic procedures involving ureteral catheterization, that the fraction of the injected dose excreted can be determined easily and rapidly. However, for general clinical application it has the disadvantages that accurate collections of urine are just as essential as in the urea clearance, and yet the results are less quantitative. For these reasons the authors consider it to be not a good test of kidney function for general clinical use.

The time of appearance and the intensity of the shadow seen on roentgenograms after the injection of Diodrast for intravenous pyelography provide a similar though even less quantitative estimate of kidney function which nevertheless is sometimes useful in small infants.

Measurement of Concentration of Substances in Blood

The one method used most commonly at present in the attempt to evaluate kidney function clinically is probably measurement of the concentration of blood urea nitrogen (BUN) or of nonprotein nitrogen (NPN). Of these two measurements, the blood urea nitrogen is more meaningful, since it measures a single substance. The results are interpreted as providing estimates of the urea clearance and therefore paralleling changes in the glomerular filtration rate. Interpreting the blood urea nitrogen in this way is based on two assumptions: (1) that the rate of excretion of urea by the kidney is constant and (2) that the urea clearance maintains a constant relationship to the inulin clearance.

If the nonprotein nitrogen is used, it is assumed further that the blood urea nitrogen represents a constant proportion of the nonprotein nitrogen. However, the rate of excretion of urea by the kidney, and therefore the blood urea nitrogen, is markedly influenced by the protein content of the diet, and neither the ratio of the clearance of urea to the clearance of inulin nor the ratio of blood urea nitrogen to nonprotein nitrogen is constant.

Measurement of the blood urea nitrogen or nonprotein nitrogen affords, at best, only a rough approximation of glomerular filtration rate. Although the results have some clinical usefulness as an indication of the presence or absence of severe reduction in kidney function, they cannot be interpreted as valid estimates of minimal or even moderate changes in kidney function.

Serum creatinine is less affected by the protein content of the diet than is the blood urea nitrogen, and measurement of changes in serum creatinine concentration has been suggested as a better estimation of kidney function.[1] However, in our judgment, measurement of serum

creatinine still affords such a rough estimation of kidney function that it has no great advantage over measurement of the blood urea nitrogen.

Estimation of Maximum Concentrating Capacity

Under most conditions, measurement of the specific gravity of the urine provides an approximate estimate of the concentration of osmotically active urinary solutes. Although other factors (glomerular filtration rate, rate of solute excretion) are concerned,[12] the maximum concentration of urinary solutes is determined primarily by the rate of tubular absorption of water under the influence of the antidiuretic hormone (ADH). Measurement of the specific gravity of the urine following moderate periods of water deprivation provides an estimate, therefore, of the maximum rate of tubular absorption of water. In the presence of normal hormonal (ADH) function this test represents a useful clinical evaluation of this kidney function.

Examination of Urine for Protein and Blood Cells

Examination of untimed specimens of urine collected without preparation of the patient serves as an important screening method for detecting, among other things, abnormal quantities of protein or blood cells in the urine. Microscopic examination of a freshly voided uncentrifuged specimen of urine is, indeed, the best method for identifying the presence of a urinary tract infection. If, however, one is interested in changes in rates of excretion of protein or red blood cells in a patient with kidney disease, it is essential to measure the rate of excretion (quantity per unit of time) rather than the concentration in urine (quantity per volume of urine). This principle has been emphasized by Addis[1] and forms the basis of the Addis count, in which the quantity of protein and the number of blood cells excreted per twelve hour night specimen are counted. The authors believe that the Addis count can and should be used clinically in observing children with certain types of kidney disease.

USE OF KIDNEY FUNCTION TESTS IN COMMON KIDNEY DISEASES OF INFANTS AND CHILDREN

Congenital Anomalies of the Kidney

Kidney function may be markedly reduced from the time of birth in infants with some congenital anomalies of the kidney (hypoplasia, congenital hydronephrosis). If kidney function is so reduced that it is responsible for failure of an infant to thrive shortly after birth, recognition of reduced function is usually not difficult. Examination of the

urine may reveal no abnormalities (increased protein or cells). However, unless the infant is severely dehydrated or is receiving an extremely high protein diet, the presence of a high blood urea nitrogen or nonprotein nitrogen will usually be sufficient to establish the existence of markedly reduced kidney function. Persistence of a high blood urea nitrogen or nonprotein nitrogen after hydration and when the infant is receiving a diet of usual protein content provides almost conclusive evidence of reduced kidney function in such an infant. A blood urea nitrogen or nonprotein nitrogen that is normal or only slightly elevated is more difficult to evaluate in such an infant in that it may be due to less marked reduction of kidney function or to a low protein intake. Persistence of a normal blood urea nitrogen or nonprotein nitrogen or return of slightly elevated values to normal while such an infant receives a diet of normal protein content would make it unlikely that his failure to thrive was due to reduced kidney function.

Although more accurate evaluation of kidney function is needed in such infants, clinically applicable tests are not available at present. If intravenous pyelograms are done, the time of appearance and intensity of the shadow seen on roentgenograms may be helpful, although, even in normal newborn infants, there is delay in the time of appearance and reduced intensity of the shadow due to immaturity of kidney function. If ureteral catheterizations are done, the phenolsulphonphthalein test can be used to compare the function of the two kidneys.

Estimation of kidney function in older children with anomalies of the urinary tract is often clinically important. The extent and progress of the disease as well as the effectiveness of treatment may have to be determined primarily by tests of kidney function. The urea clearance is the most useful test for detecting relatively minor changes in function in these children.

Acute Nephritis

Tests of kidney function have important application in the evaluation of children with acute nephritis. In our experience the two most useful tests are the urea clearance and the Addis count. If the urine is grossly bloody at the onset, an Addis count is not needed. However, the diagnosis of acute nephritis may depend upon interpretation of the significance of a "few" red blood cells or a "trace" of protein in a routine urine. In these instances the correct diagnosis may depend upon the Addis count. Urea clearances are done at the onset of the disease and at two to four week intervals thereafter. If the urea clearance remains normal or, having been decreased, becomes normal during the first two or even three months of the disease, recovery is practically assured and additional clearance tests are not required. The Addis count, which is

usually still abnormal at this time, is repeated at one to three month intervals to determine the time of complete healing.

Nephrosis

In our experience the urea clearance is the most useful of the clinically applicable tests for evaluating kidney function in children with nephrosis. Early in the course of nephrosis the ultimate outcome cannot be predicted from any tests of kidney function.[3] However, the presence of normal kidney function at any stage indicates that recovery may occur irrespective of the duration of the disease. On the other hand, reduced function persisting over a period of many months indicates less hope for eventual recovery. In this sense, knowledge of the status of kidney function during the course of nephrosis provides some prognostic aid. In this Clinic, urea clearances are done in children with nephrosis at intervals of three to six months. Addis counts, which are done at each visit, are used to follow changes in rate of protein excretion. Such changes have no prognostic significance, but may herald the onset of exacerbations or remissions.

Acute Renal Failure

In the presence of oliguria in a dehydrated child who may also be suspected of having acute renal failure, determination of the specific gravity of the urine is of great diagnostic importance. If the oliguria is due to dehydration, the specific gravity is high, whereas at the onset of acute renal failure the urine characteristically is dilute with a low specific gravity. The rate of return of kidney function in a child recovering from acute renal failure is judged best by serial determinations of the urea clearance and of the maximum concentrating ability.

APPENDIX

Procedures and Methods

The following procedures and methods are used currently in the Department of Pediatrics of The New York Hospital-Cornell Medical Center:

Measurement of Discrete Kidney Functions. The procedures and methods used in the more refined kidney function tests are described by Barnett, Hare, McNamara and Hare[5] and Mattar, Barnett, McNamara and Lauson.[14]

Urea Clearance. Children having clearances done in the outpatient department are instructed to take 10 ml. of water per kilogram one hour and again one-half hour before arriving. A light breakfast is per-

mitted, although foods containing a high content of protein are excluded. On arrival, the time of emptying the bladder is noted and two timed urine collection periods ranging from twenty minutes to one hour (depending on the rate of urine flow) are collected. Blood is drawn at the end of the second period rather than between the periods to avoid the antidiuresis which frequently follows a venipuncture. Urea is determined in blood and urine by the method of Archibald.[2]

The clearance of urea in children of different height and weight is calculated as follows:

DATA FROM LABORATORY

Blood urea nitrogen (BUN) mg./100 ml.
Urine urea nitrogen (U) mg./100 ml.
Urine volume (in ml.)

MEASUREMENTS REQUIRED

Height
Weight

CALCULATION

1. Ideal weight:* calculated from height and age from appropriate table (R. M. Woodbury[20])
2. Surface area (S.A.): calculated from height and ideal weight* from nomogram (E. F. DuBois[7])
3. Surface area factor (f S.A.) $= \frac{1.73\dagger}{\text{S.A.}}$
4. Urine flow per min. (V) $= \frac{\text{urine volume (ml.)}}{\text{duration of period (min.)}}$
5. Rate of urine flow corrected for surface area (V_{COR}) $= V \times$ f S.A.
6. If V_{COR} is greater than 2 ml. per min.:
   Clearance (C_U) $= \frac{U \times V_{COR}}{BUN}$; and $\frac{C_U}{75\ddagger} \times 100 =$ % of average normal
7. If V_{COR} is less than 2 ml. per min:
   $C_U = \frac{U \times \sqrt{V_{COR}}}{BUN}$; and $\frac{C_U}{54\S} \times 100 =$ % average normal

Creatinine Clearances. The procedures and methods are described in Mattar, Barnett, McNamara and Lauson[14] and Hare.[11]

Phenolsulphonphthalein Excretion. This is described in Ham.[9]

Addis Count and Determination of Maximum Concentrating Ability. The following instructions are given to the parents:

1. The day before the urine is to be collected, the child should have no liquids after his noon meal. If he becomes very thirsty, as small amount of fluid as possible should be given.
2. Before going to bed the child should empty his bladder as completely as possible. *Note and record the exact time of this urination, but do not save the urine.*
3. If the child has to urinate during the night, the urine is passed directly into a bottle (which has been sterilized by boiling) if the patient is a boy, or into a clean dry chamber from which every drop is poured into the bottle if the patient is a girl.

* Used only for patients with edema. For other patients, actual weight is used.
† Average value for surface area in adult.
‡ Average value for maximum clearance per 1.73 square meters.
§ Average value for standard clearance per 1.73 square meters.

The first urine passed on arising is collected in a similar way and the time of this urination is noted and recorded. Usually only this one specimen is passed if liquids have been properly restricted the day before.

4. Keep the bottle well stopped and in a cool place until it is brought to the clinic.

Note: Be sure to wash and dry a girl's vulva before collecting the urine.

Normal values for these tests and the technique for examining the urine are given in Ham.[10]

In this Clinic, normal upper limits for excretion of red blood cells and protein are taken as one million and 80 mg., respectively, per twelve hours.

REFERENCES

1. Addis, T.: Glomerular Nephritis. Diagnosis and Treatment. New York, Macmillan Company, 1950.
2. Archibald, R. M.: Colorimetric Determination of Urea. J. Biol. Chem., *157*: 507, 1945.
3. Barnett, H. L., and Shibuya, M.: Nephrosis in Children. Postgraduate Med., *15*:362, 1954.
4. Barnett, H. L., and Vesterdal, J.: The Physiologic and Clinical Significance of Immaturity of Kidney Function in Young Infants. J. Pediat., *42*:99, 1953.
5. Barnett, H. L., Hare, K., McNamara, H., and Hare, R.: Measurement of Glomerular Filtration Rate in Premature Infants. J. Clin. Investigation, *27*:691, 1948.
6. Bruck, E.: Urea Clearance Studies. Report of the Eighth M & R Pediatric Research Conference: Renal Function in Infants and Children, March, 1953, p. 18.
7. DuBois, E. F.: Basal Metabolism in Health and Disease. 3rd ed. Philadelphia, Lea & Febiger, 1936, p. 135.
8. Friederiszick, F. K.: Nieren Clearance Untersuchungen im Kindesalter. Basel, S. Karger, 1954.
9. Ham, T. H.: A Syllabus of Laboratory Examinations in Clinical Diagnosis. Cambridge, Harvard University Press, 1951, p. 274.
10. Ham, T. H.: Op. cit., p. 276.
11. Hare, R. S.: Endogenous Creatinine in Serum and Urine. Proc. Soc. Exper. Biol. & Med., *74*:148, 1950.
12. Lauson, H. D.: The Problem of Estimating the Rate of Secretion of Antidiuretic Hormone in Man. Am. J. Med., *11*:135, 1951.
13. McCance, R. A.: Renal Physiology in Infancy. Am. J. Med., *9*:229, 1950.
14. Mattar, G., Barnett, H. L., McNamara, H., and Lauson, H. D.: Measurement of Glomerular Filtration Rate in Children with Kidney Disease. J. Clin. Investigation, *31*:938, 1952.
15. Parenzan, L., and Sereni, F.: Contributo allo Studio della Funzionalita Renale della Prima Infanzia. Pediatria, *61*:665, 1953.
16. Rubin, M. I., Bruck, E., and Rapoport, M.: Maturation of Renal Function in Childhood. J. Clin. Investigation, *28*:1144, 1949.
17. Smith, H. W.: The Kidney. Structure and Function in Health and Disease. New York, Oxford University Press, 1951.
18. Vesterdal, J., and Tudvad, F.: Studies on the Kidney Function in Premature and Fullterm Infants by Estimation of the Inulin and Para-aminohippurate Clearances. Acta paediat., *37*:429, 1950.
19. West, J. R., Smith, H. W., and Chasis, H.: Glomerular Filtration Rate, Effec-

tive Renal Blood Flow and Maximal Tubular Excretory Capacity in Infancy. J. Pediat., 32:10, 1948.
20. Woodbury, R. M.: Statures and Weights of Children under Six Years of Age. U.S. Department of Labor, Children's Bureau Publication No. 87, 1921.

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TESTS OF EXOCRINE FUNCTIONS OF THE PANCREAS IN CHILDHOOD

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The conditions commonly affecting the pancreas are noteworthy because of a remarkable age predilection. Except for the occurrence of pancreatic achylia as a feature of the generalized disease mucoviscidosis, there are relatively few conditions in which pancreatic function is severely and irreversibly disturbed in childhood. Mucoviscidosis predominantly affects infants and children, yet with the increased awareness of the disease, improvement in diagnostic methodology and a broadening of the diagnostic concepts a number of adults are now recognized as having this disease. Acute and chronic pancreatitis, carcinoma of the pancreas, acquired cysts, calcification of the pancreas, and pancreatic duct obstruction due to stenosis are conditions rarely encountered in childhood. Malformations, which in general are detected in early life, may go unrecognized when there is no interference with the function of the organ. Even in the case of the annular pancreas not all possessors show signs of intestinal obstruction. However, when severe functional impairment occurs as in agenesis or congenital hypoplasia, life may terminate early. Failure of both internal and external secretions of the pancreas may be evident in these cases. It is of interest to point out that even with extensive damage to the pancreas with almost complete replacement fibrosis, as occurs in long-standing cases of mucoviscidosis, the islet function is not sufficiently interfered with to produce symptoms of diabetes mellitus.

We shall limit our discussion to the external secretory activity of the pancreas as it is reflected in disease states in childhood. In areas where

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extreme dietary deficiencies are uncommon, this is tantamount to a consideration of some aspects of mucoviscidosis. Experimentally, the pancreas of animals may be damaged in a number of ways, as by infection with the Coxsackie viruses, by feeding inadequate diets or by the administration of a variety of toxic substances, including bacterial products. Vegheli[25-27] believes that many of these states have human counterparts and refers to pancreatic dysfunction in scarlet fever, epidemic parotitis, toxic states and nutritional deficiencies. The dysfunction is usually reversible and may be detected by finding a dissociation of or abnormally high or low values for pancreatic amylase, lipase and trypsin in the duodenal fluid. In kwashiorkor, a nutritional disease commonly seen in South Africa, pancreatic function is impaired as judged by the diminished output of pancreatic enzymes.[7] It is not clear whether pancreatic dysfunction is present in all cases and to what extent the process is reversible. In our studies of a large number of infants with severe malnutrition resulting from a variety of conditions, pancreatic function as determined by amylase, lipase and protease activity is normal except in those patients with mucoviscidosis. A specific diminution in amylase activity in duodenal aspiration has been claimed in celiac disease by Andersen,[1] a finding we are unable to confirm.

DIRECT TESTS OF PANCREATIC FUNCTION

The determination of pancreatic function is difficult for various reasons. In the first place the reserve power of the pancreas is great; as little as 10 to 15 per cent of the organ may be present without evidence of impaired function. Studies of the concentration of digestive enzymes before and after partial extirpation of the pancreas in children[9] and in experimental dogs[7] amply confirm this statement. Andersen[1] pointed out that clinical symptoms of pancreatic failure resulted only when 90 per cent or more of the pancreas was involved in her series of patients with pancreatic fibrosis.

Duodenal Fluid Analysis

The most direct method of studying pancreatic function—by analyzing duodenal fluid for digestive enzymes—is a procedure which is inconvenient for the patient, time consuming and difficult for the house officer and too often not sufficiently routinized for the clinical laboratory to perform with the same degree of assurance as other procedures. Even when all these obstacles are overcome, we still have the problem of drawing conclusions from a fluid which may vary considerably from one moment to the next. The duodenal fluid is a mixture of pancreatic

secretion, bile, duodenal secretion and, perhaps to a slight extent, gastric contents.

The wide range of activity reported as occurring in normal children is a reflection of (1) varying activity of the gland, (2) the extent of the contribution of pancreatic secretion to the total mixture, (3) the technique or method of collection, the time interval between the collection of the specimen and the analyses, and finally (4) the methods of assay.

Although there is no substitute for experience in the collection and assay, the following suggestions are offered. The physician collecting the fluid should provide an adequate specimen for examination. He should be familiar with the methods of assay, the approximate wide range of normal activity and the factors influencing this activity. Armed with this knowledge he will not be content with entrusting anyone but himself to the task of checking the position of the tube, supervising the collection, making certain that it is collected on ice and that no delay occurs between collection and initiation of assay. Should one have doubts concerning the adequacy of the specimen, a repeat duodenal intubation should be carried out. A brief description of our procedure follows.

Technique. The fluid is obtained after a fast of approximately eight hours. The patient is well wrapped. A number 10 or 12 single lumen Levin tube is satisfactory. The duodenal tube should not be sterilized in a coiled position. After sterilization the tube should be hung on a rack and not folded. Firm rubber tubes rather than soft ones are preferred. The tube is inserted through the nostril and guided into the duodenum under direct fluoroscopic visualization. This procedure is carried out without difficulty in most instances. The collection is made into test tubes kept in an ice bath with an attempt to obtain fractional specimens at ten to thirty minute intervals. Gentle suction with a syringe or milking the tube may augment the flow, which is usually intermittent. Although three to five separate specimens are collected, one or two of the most satisfactory ones are used for analysis. Five or 6 cc. will suffice for a complete assay. In our routine assay we measure and record the following: *p*H, volume per unit of time, viscosity, bile concentration and activity of amylase, lipase and proteases. If it is impossible to carry out the various enzyme assays on the same day of collection, one may store the specimen on the deep freeze and carry out the assay in the next few days without experiencing any loss of enzyme activity.

Other Tests. At times and in special circumstances the duodenal fluid may be subjected to other tests such as microscopic examination, determination of eosinophile count, measurement of bicarbonate content or electrolyte composition.

Tests of Enzyme Activity. Should one wish to determine pancreatic reserve or pancreatic function after stimulation of the pancreas, the introduction of olive oil intraduodenally or secretin intravenously with examination of serial specimens is recommended. Figure 25 illustrates the type of information one may obtain after olive oil stimulation of the pancreas. In this case the fasting values for amylase, lipase, carboxypeptidase and trypsin were low. After olive oil stimulation a sharp

rise in the lipase and trypsin activity resulted with an insignificant rise in the other enzymes. This type of incomplete response is obtained when the pancreas is still able to function, but only when subjected to stimulation. In dealing with a normal pancreas the fasting values for

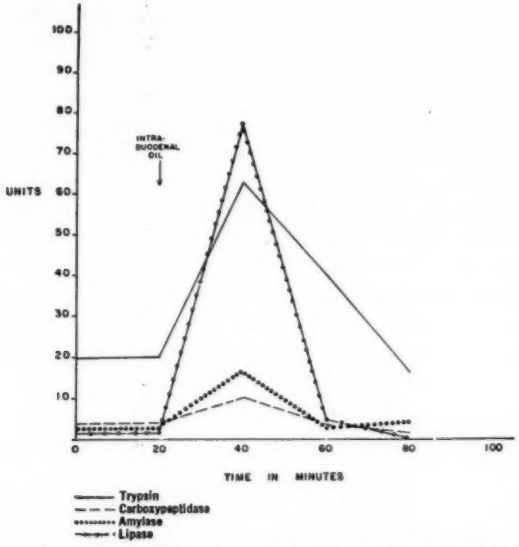

Fig. 25. Pancreatic enzyme response in patient with partial pancreatic insufficiency.

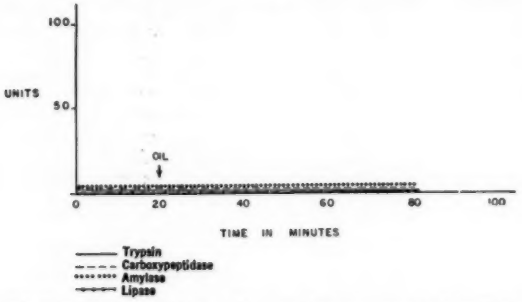

Fig. 26. Response in patient with complete pancreatic insufficiency.

the digestive enzymes are high and the stimulus of intraduodenal olive oil will increase enzyme activity almost twofold in thirty to sixty minutes with a return to prestimulation levels in about ninety minutes. In addition, there is an increase in volume as well as in alkalinity. With complete loss of pancreatic function stimulation with olive oil or intravenous secretin will not alter the zero enzyme values noted in the

fasting state, nor will there be an increase in volume or change in *p*H (Fig. 26). ***Some patients with apparent inactivity of the digestive enzymes in the fasting state may be able to secrete some if not all of the pancreatic enzymes when this organ is stimulated.*** Some of the apparent inconsistencies with the indirect tests for pancreatic function may occur in patients with absent fasting enzymes in whom the pancreas is still able to respond when stimulated. Unfortunately, it is impossible to predict which cases with zero fasting values will or will not respond to the pancreatic stimulus.

TABLE 11. *Duodenal Fluid Findings*

TEST	METHOD	HEALTHY INFANTS AND CHILDREN	CELIAC DISEASE	MUCOVISCIDOSIS	
				Complete Pancreatic Insufficiency	Partial Pancreatic Insufficiency
Volume		8–25cc./hr.	20–45cc./hr.	1–10cc./hr.	5–25cc./hr.
***p*H**	Indicator paper or *p*H meter	6.0–8.4	6.0–8.4	3.5–8.1	5.0–8.4
Bile	Colorimeter	20–125	15–75	10–125	10–125
Viscosity	Shwachman and Patterson[23,24]	Below 3 min.	Below 3 min.	Over 3 min. in 87% pts.	Over 3 min. in approximately 70% pts.
Amylase	Free-Myers[10]	4–20	4–20	0–1	0–20
Under 6 mos.		0	0	0	0
Lipase	Nothmann, Pratt, Benotti[17]	3–10	3–10	0–1	0–10
Protease	Free-Myers[10]	18–70	18–70	0–2.5	0–70
Carboxypeptidase*	Ravin, Seligman[19,23]	0.40–1.00	0.40–1.00	0–0.40	0.00–1.00
Chymotrypsin*	Ravin, Bernstein, Seligman[20,21]	11–65	11–65	0–5	0–65

*Based on examination of approximately 250 patients. The other values are based on a study of over 1000 patients. The numbers refer to range of activity of the enzymes as defined in references given and as determined in our laboratory.

The duodenal fluid findings in three groups of children are shown in Table 11. Patients with mucoviscidosis are separated in this discussion into two groups on the basis of pancreatic function. In the past the only cases generally recognized were those with complete pancreatic insufficiency, a diagnosis simple to make. We have applied the term "partial pancreatic insufficiency" to those patients in whom definite evidence of dysfunction exists. The recognition of this entity is difficult and at times can be made only in retrospect.* The usual procedure of examining the duodenal fluid for protease activity will not detect the majority of these patients. Frequently serial studies of pancreatic function in these patients show progressive loss of function. This entity is of clinical significance because extensive pulmonary involvement may be present with only minimal pancreatic disturbance. Some of the clinical and laboratory clues suggestive of this entity may be typical pulmonary symptomatology, a positive family history and the finding of increased duodenal fluid viscosity and perhaps a diminution in activity of one or more pancreatic enzymes or an abnormal response of the pancreas to intraduodenal olive oil stimulation. One notes in Table 11 that the volume of fluid per unit of time is markedly diminished in patients with complete pancreatic insufficiency in contrast to the increased volume in celiac disease. The *p*H of the duodenal fluid in patients with complete pancreatic insufficiency may be lower than normal, owing to the failure of the pancreas to secrete bicarbonate.

Bile concentration is measured in the colorimeter. This measurement is of special importance in the study of patients suspected of having obstructive jaundice.

The *viscosity measurement* is useful because it may be the only abnormality detected in the duodenal fluid in patients in whom full-blown pancreatic enzyme deficiency develops. In 87 per cent of 250 patients with mucoviscidosis the duodenal fluid viscosity was over three minutes. The value in healthy and nonpancreatic infants and children varies from one to one and one-half minutes, or approximately two to three times that of distilled water. Occasionally the viscosity is elevated in other conditions which are rarely if ever considered in the differential diagnosis of mucoviscidosis. For various reasons the viscosity measurement could not be made in approximately 10 per cent of the patients with mucoviscidosis. The determination requires approximately 3 cc. of fluid and is made at 0° C. in an Ostwald viscosimeter previously standardized with distilled water at 20° C. to a flow time of thirty-two or thirty-three seconds. We have used a variety of methods for determining enzyme activity, and the ones recorded in Table 11 seem to be the most practical for our routine assay.

*The recent development in our laboratory of a simple method to detect the abnormally high sodium and chloride content in thermal sweat, which is characteristic of patients with mucoviscidosis, appears to be helpful in detecting these patients.

Amylase is measured by the Free-Myers method, which is based on the amount of reducing substance liberated from a starch substrate.[10] The substrate for lipase is olive oil, and the technique is that of Nothmann, Pratt and Benotti.[17] *Protease* is measured by the Free-Myers method, using casein as a substrate.[10] We find this method or the gelatin viscosity test recently devised by Leubner and Shwachman[12] more useful in detecting gradations of activity than the simple Andersen-Early method.[2] The *carboxypeptidase* and *chymotrypsin tests* as developed by Seligman and co-workers[19–21, 23] have been used by us in a study of over 250 patients. In general these tests yield values which parallel protease activity. However, when dealing with cases of partial pancreatic insufficiency, one need not get values of comparative activity. In the majority of cases, both in health and disease, the pancreatic enzymes respond in a parallel fashion. When a dissociation of enzyme values is found, such as a low to absent protease with a normal lipase and amylase or some other combination, the finding is sufficiently unusual to merit repeat duodenal study to verify the finding. We have noted dissociation of enzyme activity in some patients with mucoviscidosis with partial insufficiency who are on the way to further loss of the remaining activity. We should like to point out that when complete loss of enzyme activity occurs, we are dealing with a permanent and irreversible condition. A fuller discussion of the methods used and interpretation of the findings listed in Table 11 has been presented.[23]

INDIRECT TESTS OF PANCREATIC FUNCTION

Although the direct tests for pancreatic function involve a great deal of work and discomfort to the patient, one should consider these measurements essential in the diagnosis of mucoviscidosis. Perhaps we will modify this position as other specific tests for this disease become practical.* At times it may be impossible to carry out the duodenal intubation, and under these circumstances one may resort to the so-called indirect tests of pancreatic function. Many of these tests refer to the faulty digestion which occurs when the pancreatic enzymes are lacking.

Stool Examination

The gross, microscopic and chemical examinations of the stools reflect this nutritional defect. Actually, one of the oldest tests is the *Schmidt test* (1904). This consists in feeding beef in a muslin sac, recovering it from the stools and examining the degree of nuclear preservation. Another old test is the *sajodin test* of Winternitz, which depends upon the splitting of an iodized oil in the intestines and the subsequent

* See footnote on page 206.

elimination of iodine in the urine. This test is not considered reliable because fats can be split in the intestines in the complete absence of pancreatic juice.[13, 23] Some of the earlier work on the presence of pancreatic enzymes in the stool concluded that these tests were useless. However, Shwachman et al.[24] have devised a simple technique for the demonstration of proteolytic activity in the stool which serves as a useful screening test for the presence of pancreatic protease.

Absorption Tests

A variety of procedures have been described to determine faulty protein digestion. We use the *gelatin absorption test* as described by Christensen and Shwachman[6] as follows:

After an 8 to 9 hour fast the patient is given Knox gelatin by mouth (1.74 gm. per kilogram with 40 gm. as the maximal dose). The gelatin is previously dissolved in hot water. The plasma glycine nitrogen is determined in the fasting specimen and in a one and one-half-hour postprandial specimen. In healthy persons the rise in glycine nitrogen is at least two and one-half times the fasting value. In pancreatic insufficiency the rise is less than twofold.

Other methods of studying protein absorption involve the feeding of iodine^{131}-labeled casein and measuring the amount of isotope recovered in urine and feces.[5] One of the classical methods of study is by way of the metabolic balance.[22] These last two methods require special skill and equipment and are too time consuming and costly to be generally available.

A number of simple tests have been devised to test fat digestion and absorption. Unfortunately a great variety of nonpancreatic conditions may yield abnormal findings, a situation not encountered in the gelatin absorption test. However, the defect in fat metabolism is so great in complete pancreatic insufficiency that most other conditions likely to show defects in absorption show them to a less marked degree. One of the earliest and most useful tests is the *vitamin A absorption test*.[3, 16]

A test dose of Oleum Percomorphum is given by mouth (0.1 cc. per pound of body weight), and the four or five hour level of vitamin A in the serum is compared with the fasting level.[18] In pancreatic insufficiency the fasting vitamin A value is low and there is little or no rise in the four or five hour level. When a water-miscible preparation is given or when pancreatin is given along with Oleum Percomorphum, a rise in the serum vitamin A level occurs.

The chylomicron technique used by Frazer et al.[8] and by Crocker in our laboratory shows a marked depression of the postabsorption hyperlipemia in patients with pancreatic insufficiency. The postprandial rise of esterified fatty acids was studied by Hirsch et al.,[11] who found a slight to no rise after fat ingestion in patients with pancreatic insufficiency.

The activity of serum amylase and lipase has been used as an indication of pancreatic function in cases of suspected acute pancreatitis, a

condition seldom encountered in children. In a limited number of serum amylase and lipase determinations the values were normal in patients with complete pancreatic insufficiency. Of interest is the recent study by MacFarlane[15] on the lowered antithrombin levels in the serum of patients with mucoviscidosis.

Tests of Clinical Response

Although we have focused our attention on laboratory tests for pancreatic function, we should not omit the clinical trial. The administration of a potent pancreatic preparation to a patient with pancreatic insufficiency may produce a dramatic clinical response. This consists in a reduction in size and number of movements with an improvement in odor and consistency. A weight gain may occur in spite of a reduction in the total caloric intake. When the substitution therapy is discontinued, a clinical relapse may take place. Rarely this favorable response

TABLE 12. *Tests for Pancreatic Function*

I. DIRECT
- Duodenal Fluid
  - Volume before and after stimulation
  - Bicarbonate or *p*H } before and after stimulation
  - Viscosity
  - Enzyme activity (amylase, lipase, proteases)
  - (1) Fasting state
  - (2) After stimulation
    - (a) Intraduodenal olive oil
    - (b) Intravenous secretin

II. INDIRECT
- A. Metabolic balance
  - Nitrogen and fat intake and output
- B. Stool examination
  - (1) Gross examination (see A above)
    - Excess bulk with increase in total fat and nitrogen
  - (2) Microscopic examination
    - Fat
    - Starch
    - Proteins (Schmidt test)
  - (3) Enzymes—trypsin
- C. Absorption tests
  - (1) Fat
    - (a) Cream
      - Chylomicron counts
      - Fatty acids
    - (b) Vitamin A esters vs. alcohol and aldehyde serum levels of vitamin A
  - (2) Proteins
    - (a) Gelatin or casein absorption tests
    - (b) I^{131}-labeled casein
- D. Clinical response
  - (1) To dietary adjustment
  - (2) To an adequate potent pancreatin preparation

is not noted in some patients with complete pancreatic insufficiency. The tests referred to in this discussion are listed in Table 12.

REFERENCES

1. Andersen, D. H.: Cystic Fibrosis of the Pancreas and Its Relation to Celiac Disease. Am. J. Dis. Child., *56*:344, 1938.
2. Andersen, D. H., and Early, M. V.: Method of Assaying Trypsin Suitable for Routine Use in Diagnosis of Congenital Pancreatic Deficiency. Am. J. Dis. Child., *63*:891, 1942.
3. Breese, B. B., Jr., and McCoord, A. B.: Vitamin A Absorption in Celiac Disease. J. Pediat., *15*:183, 1939.
4. Brock, J. F., and Autret, M.: Kwashiorkor in Africa. World Health Organization Monograph Series No. 8, Geneva, 1952.
5. Chinn, A. B., Lavik, P. S., Stitt, R. M., and Buckaloo, G. W.: Use of I^{131} Labelled Protein in the Diagnosis of Pancreatic Insufficiency. New England J. Med., *247*:877, 1952.
6. Christensen, H. N., and Shwachman, H.: Determination of the Plasma Glycine after Gelatin Feeding as a Diagnostic Prodecure for Pancreatic Fibrosis. J. Clin. Investigation, *28*:319, 1949.
7. Coffey, R. J., Mann, F. C., and Bollman, J. L.: III. The Influence of the Pancreas on the Utilization of Foodstuffs. Am. J. Digest. Dis., *7*:144, 1940.
8. Elkes, J. J., Frazer, A. C., and Stewart, H. C.: The Composition of Particles Seen in Normal Human Blood under Darkground Illumination. J. Physiol., *95*:68, 1939.
9. Farber, S,. Shwachman, H., and Maddock, C. L.: Pancreatic Function and Disease in Early Life. I. Pancreatic Enzymes Activity and the Celiac Syndrome. J. Clin. Investigation, *22*:827, 1943.
10. Free, A. H., and Myers, V. C.: The Estimation of the Enzymes, Amylase, Proteinase and Lipase in Duodenal Contents. J. Lab. & Clin. Med., *28*:1387, 1943.
11. Hirsch, E. F., Carbonaro, L., Biggs, A. D., and Phillips, F. L.: Postprandial Hypolipemia of Pancreatic Fibrocystic Disease. Am. J. Dis. Child., *86*:721, 1953.
12. Leubner, H., and Shwachman, H.: To be published.
13. McClure, C. W.: Functional Activities of the Pancreas and Liver. New York, Medical Authors' Publishing Co., 1937, pp. 41, 42.
14. McClure, C. W., Vincent, B., and Pratt, J. H.: Absorption of Fat in Partially and Completely Depancreatized Dogs. J. Exper. Med., *15*:381, 1917.
15. MacFarlane, J. C. W.: Serum Antithrombin Level in Fibrocystic Disease of the Pancreas. Lancet, *2*:311, 1952.
16. May, C. D., Blackfan, K. D., McCreary, J. F., and Allen, F. H., Jr.: Clinical Studies of Vitamin A in Infants and in Children. Am. J. Dis. Child., *59*: 1167, 1940.
17. Nothmann, M. M., Pratt, T. D., and Benotti, J.: The Effect of Ligation of the Pancreatic Ducts and of Pancreatectomy after Duct Ligation on Serum Lipase. J. Lab. & Clin. Med., *33*:833, 1948.
18. Pratt, E. L., and Fahey, K. R.: Clinical Adequacy of a Single Measurement of Vitamin A Absorption. Am. J. Dis. Child., *68*:83, 1944.
19. Ravin, H. A., and Seligman, A. M.: The Colorimetric Estimation of Carboxypeptidase Activity. J. Biol. Chem., *190*:391, 1951.
20. Ravin, H. A., Bernstein, P., and Seligman, A. M.: A Colorimetric Micromethod for the Estimation of Chymotrypsin Activity. J. Biol. Chem., *208*:1, 1954.
21. Richmond, R. C., and Shwachman, H.: Studies in Mucoviscidosis, Chymotrypsin Activity of Duodenal Fluid. To be published.
22. Shohl, A. T., May, C. D., and Shwachman, H.: Nitrogen and Fat Metabolism in Infants and Children with Pancreatic Fibrosis. J. Pediat., *23*:267, 1943.

23. Shwachman, H., Leubner, H., and Catzell, P.: Mucoviscidosis, in Levine, S. Z., Ed.: Advances in Pediatrics. Chicago, Year Book Publishers, Inc., 1954, Vol. II.
24. Shwachman, H., Patterson, P. R., and Laguna, J.: Studies in Pancreatic Fibrosis. A Simple Diagnostic Gelatin Film Test for Stool Trypsin. Pediatrics, 4:222, 1949.
25. Veghelyi, V. P.: Pancreatic Function in Scarlet Fever. Pediatrics, 4:94, 1949.
26. Veghelyi, P. V., Kemeny, T. T., Pozsonyi, J., and Sos, J.: Dietary Lesions of the Pancreas. Am. J. Dis. Child., 79:658, 1950.
27. Veghelyi, P. V., Kemeny, T. T., Pozsonyi, J., and Sos, J.: Toxic Lesions of the Pancreas. Am. J. Dis. Child., 80:390, 1950.

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ENDOCRINE FUNCTION TESTS IN PEDIATRIC PRACTICE

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It is manifestly impossible even to list in this review all the various laboratory procedures used in the diagnosis and evaluation of a patient with an endocrinopathy. Some tests, therefore, will not be discussed at all, and others, especially those well known for their use in nonendocrine disease, will only be touched upon. The text is concentrated upon simple representative tests of function which are ordinarily available even in the absence of a full-time investigator in the endocrine field. There will be no discussion of bio-assays except for gonadotropin assays, since, in general, these require special care and are expensive for the routine laboratory. Tests for one of the most important endocrinopathies, diabetes, will not be discussed in this report because this field is well known to the practitioner of pediatrics.

Endocrinologists place more reliance upon laboratory procedures in both their clinical and their investigative work than do clinicians in most other fields. It is only fair to state, however, that these procedures, though necessary and important, are no more valid than any other laboratory tests. One must realize that a single laboratory test cannot be relied upon for diagnosis. Many of the procedures to be discussed are helpful and have been refined to a high degree of reliability, but when the clinical diagnosis is equivocal, the laboratory results unfortunately also tend to be equivocal. Even in the field of endocrine function one must be resigned to the fact that the day is not at hand when properly processed samples of blood or urine will take the place of careful clinical evaluation of the patient in making the diagnosis and guiding the treatment.

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TESTS OF ANTERIOR PITUITARY FUNCTION

Gonadotropin Excretion

Abnormalities in pituitary function are most easily recognized, at present, by measuring dysfunction of its target glands. However, a few more or less direct estimations of pituitary function can be made. Probably the most important direct assay is the measurement of gonadotropin excretion in the urine. These materials are not present in the urine in measurable amounts until pubescence. The method of Klinefelter and Albright[11] is still the best and simplest method for clinical use. More precise quantitation can be achieved by measuring uterine weights and the like, but for clinical purposes the original method seems satisfactory. Bio-assay measurements for pituitary adrenocorticotropic and thyrotropic hormones are also possible, but at present these are experimental laboratory procedures and are not the simple, cheap, routine test that the measurement of gonadotropin can be.

In addition to its general importance as an index of one pituitary function, the measurement of gonadotropins is of special clinical importance in the syndrome of *ovarian agenesis*. Patients with this syndrome may have certain congenital anomalies such as coarctation of the aorta, webbed neck and polydactylism; they are dwarfed and have rudimentary or absent ovaries. In the presence of all the congenital anomalies the diagnosis is readily considered. In the absence of the anomalies, however, an etiologic diagnosis is hard to make in the case of a short girl who at thirteen has not shown maturation. If the patient has ovarian agenesis, the gonadotropin level in her urine will be extremely high. Treatment with estrogen causes the gonadotropin level to fall in these girls as it does in the postmenopausal patients so treated. The finding of a high gonadotropin excretion in a girl who is somewhat dwarfed and sexually immature at the age of puberty or beyond comes as close as any single laboratory finding ever does to being diagnostic. Patients with idiopathic late sexual maturation, or infantilism due to pituitary deficiency, of course have little or no gonadotropin excretion in the urine.

Glucose and Insulin Tolerance Tests

The disturbances in carbohydrate metabolism accompanying pituitary deficiency are the basis for the use of glucose and insulin tolerance tests in the diagnosis of *hypopituitarism*. The patient with hypopituitarism has difficulty in maintaining a normal blood sugar level and is unable to recover easily from induced hypoglycemia. The details of these tests

are well known, but it should be pointed out that in any suspected case of hypopituitarism an insulin tolerance test should be done with half the usual dose of insulin. In other words, the dose should be 0.05 unit per kilogram intravenously rather than 0.1 unit per kilogram.

Though the so-called insulin tolerance test is more often helpful, in many instances the same hypofunction can be demonstrated by a regular glucose tolerance test carried out for five hours. After the usual initial rise in glucose levels the expected drop slightly below the fasting level may be accentuated, so that the blood sugar level falls low enough to give hypoglycemic symptoms. The expected rebound, after the fall in blood sugar below the fasting level, is delayed or may not occur.

Growth Hormone Production

Most pediatric clinical problems concerning the pituitary are those of *hypofunction*. While there is no direct measurement of pituitary growth hormone available, the end result of the lack of this hormone is retarded growth, of course. Dwarfing may be due to craniopharyngioma, rarely to chromophobe adenoma of the pituitary or, more frequently, to panhypopituitarism of unknown origin. The roentgenographic appearance of the region of the sella is of help in distinguishing these conditions. In addition, the secretion of growth hormone by the pituitary is presumably depressed during chronic debilitating illnesses, though investigation of this point is handicapped by the lack of a direct assay method for this material. It is interesting that patients with congenital cyanotic heart disease and retardation of growth also have delayed sexual maturation associated with late appearance of gonadotropins in the urine. This would suggest that the retarded growth and sexual development are not due as much to lack of response of the poorly oxygenated tissues to normal amounts of pituitary hormones as to actual suppression of these hormones by the effects of the heart disease.

Serum Phosphate Level

The level of serum phosphate is a crude index of growth activity and is high in the normal child while in a period of active growth and in patients with acromegaly. This is probably not a very helpful factor in diagnosing the rare case of *pituitary gigantism* in childhood. It is enlightening, however, in conjunction with bone age, in determining when growth is at an end in the normal adolescent girl whose mother is worried about her growing too tall.

TESTS OF THYROID FUNCTION

Protein-Bound ^{131}Iodine Level

The measurement of the basal metabolic rate is still a satisfactory method of estimating thyroid activity. In very young children, however, it is frequently difficult to establish a truly basal rate. In infants, indeed, it is impossible with the usual apparatus. It is no wonder, then, that recently introduced methods of measuring thyroid activity have been accepted with alacrity by pediatricians. More and more laboratories are equipped to measure either protein-bound iodine or butanol-extractable iodine. These are good indices of the circulating thyroid hormone at the time the blood is obtained. The methods require great skill and are fairly time consuming and tedious. At present, however, they constitute probably the best single index of thyroid function for pediatric practice. Occasionally, obviously hypothyroid patients are seen who have protein-bound iodine levels within the normal range. The chief difficulty with the method is found in those patients who have equivocal hyperthyroidism or hypothyroidism. The former is the more common problem, and in these patients a protein-bound iodine at the higher end of the normal range, or just above the expected value for normal, is frequently found.

The intake of iodine by the patient before the blood specimen is obtained for the measurement of protein-bound iodine leads to erroneously high values. Therefore blood for protein-bound iodine measurements should be obtained before intravenous pyelograms, bronchograms, and so on, are carried out. An effort should also be made to make sure that there has been no previous ingestion of iodine in various therapeutic mixtures. A measurement of total iodine along with the protein-bound iodine will determine those patients who had received iodine. Incidentally, the protein-bound iodine level may remain high for months after the various diagnostic procedures which utilize iodine for the sake of its radiopacity.

Radioactive Iodine Uptake

The second new method for estimating thyroid activity is the measurement of radioactive iodine uptake by the gland. Small amounts of radioactive iodine are administered by mouth or intravenously and the concentration of the material measured over the gland. In older children the excretion in the urine may also be measured. Equivocal results are sometimes obtained by this method as well, since the extremes of radioactive iodine uptake of the normal and the hypothyroid or the normal and the hyperthyroid overlap. In addition, when a patient has a large thyroid mass such as seen in certain goitrous cretins, the radioactive iodine uptake is rapid and large, and is not helpful in diagnosis of thyroid hypofunction.

Combinations of these two new methods giving measures of conversion of radioactive iodine to protein-bound radioactive iodine are a great advance on either of the methods alone. However, this can be carried out only in laboratories specially organized for the study of thyroid problems. The combination of the methods gives information on the incorporation of iodine into thyroid hormone as well as its uptake by the gland. In addition, other details of thyroid metabolism can be studied.

Another modification of the radioactive iodine uptake study is of much more practical importance. Patients frequently are seen who have received thyroid long enough to have wiped out any of the signs or symptoms of *hypothyroidism*. Formerly the only way to establish whether such a patient actually had been hypothyroid was to stop treatment for a period of six weeks or several months. At the end of this time clinical signs of hypothyroidism and an elevated serum cholesterol would be found if the patient had actually been hypothyroid. Both hypothyroid patients and normal persons who have been receiving thyroid for some time will have a normal protein-bound iodine and a depressed radioactive iodine uptake. Now we can administer thyrotropic hormone to these two groups of patients and measure the radioactive iodine uptake again. The normal patients will demonstrate a return towards the normal radioactive uptake. The patients with hypothyroidism will not. This is a simple and practical diagnostic method which does not require waiting a minimum of six weeks for an answer. One must remember, however, that there is a certain group of cretins whose thyroids are capable of taking up radioactive iodine.

Serum Cholesterol Level

The measurement of serum cholesterol is frequently of help in hypothyroidism when it is high. There is more variation in hyperthyroidism, and it cannot be relied upon, though hyperthyroid patients often have low serum cholesterols. In the young cretin, unfortunately, the cholesterol level is normal for many months after birth. However, if this patient is treated with thyroid and the thyroid medication is then stopped, his cholesterol will rise to the high level seen in the older hypothyroid patients, be they cases of congenital or acquired hypothyroidism.

Creatine Excretion

In hypothyroidism creatine excretion is depressed and in hyperthyroidism increased. This is a helpful ancillary method of diagnosis, but it is not used often as a practical tool.

Skeletal Maturation

Skeletal maturation is an important diagnostic aid in the patient in the pediatric age group who is suspected of thyroid disorder. In hyperthyroidism there is a slight advancement in bone age that occurs early, though this is obscured later by the lack of growth that goes with the increasing debility. The bone age in hyperthyroidism is therefore usually not of great practical value. In hypothyroidism, on the other hand, it is extremely helpful. It must be remembered that in dwarfing of nearly every sort in childhood, the bone age is retarded, but it is retarded in proportion to the retardation in growth. In hypothyroidism skeletal maturation is even more retarded than is physical growth. This may be restated simply in the terms popularized by Wilkins. In hypopituitarism and most other causes of growth retardation the bone age is equivalent to the height age. Height age here represents that age for which the patient's height would be at the fiftieth percentile. In hypothyroidism the bone age is retarded beyond the retardation in height age.

Thyroid Medication

The final test of hypothyroidism in children is the clinical response to thyroid medication. As far as is known there is no harm in the administration of the thyroid as a therapeutic test. Patients with normal thyroids have received exogenous thyroid for many years in amounts sufficient to suppress their own thyroid activity, without loss of the ability of their thyroids to respond normally when thyroid medication was stopped or when thyrotropic hormone was administered. On the other hand, this procedure has been much abused, and patients have received thyroid for long periods of time needlessly. This is harmful because other causes of the patient's trouble are not sought for. In retarded children hope of improvement is implied by the continued administration of any medicine. When these illusory hopes finally disappear, the patient and family often are worse off emotionally than they would have been had hope not arisen in the first place. If the patient is hypothyroid, he should respond dramatically to administration of thyroid within a month of beginning treatment. The changes are rapid and gratifying. Indeed, the mother of the young treated cretin is one of the most grateful parents in pediatric practice.

TESTS OF ADRENAL CORTICAL FUNCTION

Tests of adrenal cortical function must be divided into three groups, depending upon which of the three major functions of the adrenal the tests chiefly measure. We will first consider tests measuring the sodium-retaining function of the adrenal cortex.

Sodium and Potassium Concentration

The simplest estimate of the desoxycorticosterone-like action of the adrenal cortex on the kidney is the measurement of sodium and potassium concentration of the blood and the content of these electrolytes in the urine. However, the concentration of sodium and potassium in the blood of the patient with early adrenal insufficiency may be normal. The characteristic picture in *Addison's disease* is, of course, a low serum sodium and a high serum potassium. This occurs in other conditions as well as adrenal insufficiency, notably in various forms of renal disease. The knowledge of the content of these electrolytes in the urine is of little value unless the intake is known or unless it is known that a simultaneous blood level of sodium is low or level of potassium is high. Thus, while it is simple in any laboratory equipped with a flame photometer to make these measurements, these values by themselves are usually not adequate to make a diagnosis of adrenal disease. In *hyperadrenocorticism* the sodium level may be high in the serum and the potassium conversely tends to be low, but this is not a universal finding and is not of much help in diagnosis, though it is of confirmatory value when found.

Measurements of sodium and potassium in the serum and urine are of more value when a provocative test of adrenal function is done by the administration of adrenocorticotropic hormone. With the administration of ACTH for twenty-four or forty-eight hours the sodium content of the urine should drop and the potassium excretion should increase. If the pretreatment electrolyte level is normal, it will change little; if it is abnormal, however, it should tend to revert towards the normal. Unfortunately, this is of no value in newborn infants, who have a paradoxical excretion of sodium when they are given ACTH. This paradoxical excretion of sodium on the administration of ACTH is of value, however, in the diagnosis of patients with *congenital adrenal hyperplasia*, since they too excrete more sodium when given ACTH, and this is of ancillary diagnostic value when these children are more than a few months old.

At this point it is worth while to mention that one of the old tests of adrenal function of this sort, the Cutler-Powers-Wilder salt deprivation test, should not be performed on any patient really suspected of having adrenal hypofunction. It is a provocative test with the elimination of sodium from the diet and the administration of potassium. It should be used only for the final elimination of the diagnosis when it is fairly well established that the patient does not have adrenal disease. It is a dangerous test if the patient does have adrenal disease. We might also mention the Robinson-Powers-Keppler diuresis test, which probably depends more on the next function of the adrenal cortex to be discussed, but will be mentioned here, since it is a simple test involving the ability

of the patient to excrete a water load. This test is not foolproof, and renal disease and hypothyroidism, for instance, may invalidate it. Bio-assay of aldosterone or other sodium-retaining hormone in the urine is a feasible but not usually available technique.

Corticoid Excretion

The second group of adrenal function tests measure the ability of the adrenal to secrete substances of which compound F (hydrocortisone) is the prototype. The urinary excretory products of these materials are measured in many laboratories. Many different methods are used to measure what we will call the corticoids in the urine. All the methods suffer from several difficulties, and single specimens using any of the methods are not a valid basis for the diagnosis of *hyperadrenocorticism* or *hypoadrenocorticism*. Once a laboratory has set up normal standards for its particular method, and these may not be taken from the literature, these tests are helpful. However, the amount excreted in the newborn period is very small absolutely, although it is as large as that excreted by the adult in proportion to the surface area of the subjects. At these low levels of excretion the test becomes much less accurate. The rise in the excretion of these materials after ACTH administration may be taken as a good index of adrenal function. There are no well established standards for the rise in small children, and each laboratory must work out its own normal controls. Severe renal disease also will throw this test off. Patients with *Cushing's disease* do not always have a high excretion of these materials, but when the adrenal is stimulated with ACTH, the response is much greater than the normal, except in cases due to carcinoma of the adrenal. In general, however, those patients with carcinoma as a cause of Cushing's disease, and indeed many of the patients with Cushing's disease due to adrenal hyperplasia, will continuously excrete a large amount of corticoid in the urine.

The well known Thorn test indirectly measures corticoid secretion by the adrenal by measuring the fall in eosinophils after a standard amount of ACTH has been given. One difficulty is that the reaction is a sensitive one, and in the presence of small amounts of corticoid other factors cause a fall in eosinophils. However, it is a simple screening test and can be carried out in any laboratory.

Recently the level of corticoid in the peripheral plasma or serum has been measured more and more frequently. Random specimens are of no value in the diagnosis of adrenal insufficiency, since normally the levels of corticoid in the blood are variable, and at times the normal person may have no measurable corticoids. In Cushing's disease these levels tend to be high, but they are not always beyond the upper limit of the normal range. At present it would seem that one of the best

measures of adrenal function is the measurement of adrenal corticoids in the peripheral blood after the intravenous administration of ACTH. These measurements at present are being carried out only by laboratories in which there is someone investigating adrenal function, but the technique itself is not exceptionally difficult, and it is likely that in the near future this will be a routine procedure. Evidence is accumulating that this is a sensitive and valid test of adrenal secretory power. The patient with *Addison's disease, hypopituitarism* or *congenital adrenal hyperplasia* responds poorly in this test. The patient with *Cushing's disease* has a supernormal response unless his disease is due to adrenal carcinoma. This test has the advantage of requiring only several hours of the patient's time and two or three specimens. It is not difficult to perform from the laboratory viewpoint. It is a direct measurement of adrenal secretion and eliminates the need to consider many nonadrenal factors.

Ketosteroid Excretion

The third major adrenal function is the secretion of androgenic material. This is estimated by measuring the excretion of 17-ketosteroids in the urine as well as the level of 17-ketosteroids in the blood. The measurement of these materials in the blood is only being carried out on a research basis, though in time this too may become a routine measurement. At present this is farther away from this status than the measurement of the adrenal corticoid in the blood.

The measurement of 17-ketosteroids in the urine is backed by long usage, though this has the disadvantages of the other urinary measurements in that it depends somewhat on the status of renal clearance, and there is a large day-to-day variation. However, any laboratory which is concerned with helping the practitioner take care of children should be able to measure 17-ketosteroids in the urine. This is of utmost value in the diagnosis of *congenital adrenal hyperplasia.* Any patient in the newborn period who is suspected of having the signs of adrenal insufficiency should have a collection of his urine made for twenty-four hours and this sample measured for 17-ketosteroid excretion, since the most common cause of Addisonian disease in the newborn period is congenital adrenal hyperplasia. The diagnosis may be made by the finding of a high ketosteroid excretion in the presence of signs suggestive of this condition. This use alone make the test valuable. The normal values obtained in different laboratories differ, but by the usual methods any twenty-four hour excretion of more than 1 mg. of 17-ketosteroid material by an infant is considered excessive and suggestive of the diagnosis of congenital adrenal hyperplasia.

In the near-future probably many methods will be widely available

for the determination of specific steroid excretion in the urine both in the androgenic and the gluconeogenic series. However, at present they are not of clinical importance. The response to substitution therapy with cortisone or hydrocortisone or desoxycorticosterone is almost impossible to judge in any patient in whom the diagnosis of Addison's disease is not apparent for other reasons.

TEST OF FUNCTION OF THE ADRENAL MEDULLA

The clinical importance of this section is only in the determination of possible *pheochromocytomas*. Though this tumor is not especially common, it is important as a curable form of hypertension. In those patients with constant hypertension the administration of such agents as Regitine or benzodioxane will usually cause a temporary lowering of blood pressure. False negatives have been found with either drug, and if the diagnosis is considered strongly, both drugs should be used when the first drug gives a negative result. Diabenamine may be also used for this purpose, but it has a slower, longer action than the first two named drugs. In the patient with only intermittent hypertension provocative tests with materials such as histamine may be carried out. Theoretically there is some danger of producing an attack with this material, but if one of the antiepinephrine drugs is available, this is minimized. The discovery of the elevation of blood sugar that occurs with many of these tumors during attacks or paroxysms is a helpful diagnostic measure. Probably the best single diagnostic measure is the measure of catecholamines in the urine, but at present this laboratory procedure is not available in the ordinary hospital.

TESTS OF GONADAL FUNCTION

Before pubescence the necessity for doing tests for gonadal function exists in those cases of *premature puberty* of any origin. At puberty the usual problem is one of delayed pubescence or one caused by the characteristically irregular menses shortly after the menarche. It need merely be mentioned in this section that 17-ketosteroid estimations measure testicular output as well as adrenal, though numerically the contribution of the testicular androgen is much smaller than that of the adrenal androgen. On the other hand, the testicular androgen is much more potent. For this reason the measure of the total 17-ketosteroids is a poor method of estimation of testicular function. Measurements of sperm counts are rarely used in pediatric practice. Biopsy of the testes, although not a common procedure, is used more often as a measure of testicular activity and structure. Urinary content of estrogens can be

measured by a method that the ordinary laboratory is capable of doing, but this method is usually not available, since it requires a photofluorometer. For clinical purposes this is usually a satisfactory method of measurement. The simplest method of measuring estrogenic function is the vaginal smear. This can be carried out by anyone in his office. For the purposes of estimating the amount of sex hormone secretion this test is capable of being mastered by the average practitioner using the simplified, single Shorr[15] stain technique. This does not hold for the recognition of neoplastic cells.

A useful device in the study of patients with *hermaphrodism* or *pseudohermaphrodism* is the vaginogram. This is used to demonstrate the presence of a urogenital sinus when there is a single meatus anterior to the anus. A catheter is inserted through the meatus and into the bladder and then radiopaque dye is injected *around* the catheter. If the catheter blocks the entrance into the bladder, the dye tends to run back into the vagina, producing a characteristic picture in which at times the cervix may be seen protruding into the vagina.

In all cases of *intersexuality* or true *hermaphrodism* final understanding of the anatomy and possible later gonadal function cannot be made by any laboratory test or combination of tests. Exploration and biopsy of the gonads are the only possible solutions to this problem at present. Recently a method of differentiation of genetic sex has been described using skin biopsies. If the work of the original authors proves reproducible in other hands, this will be of great value in determining the genetic sex, but it will not show what hormones the gonads in the patient with intersexuality will produce at puberty, nor what the gonads actually are.

TESTS OF POSTERIOR PITUITARY FUNCTION

In this section only tests for the diagnosis of *diabetes insipidus* will be considered. The basic tests here are the old ones of the measurement of the urine concentration after thirsting and after the administration of Pitressin. However, there is one simple test that can be carried out which helps establish diabetes insipidus and rules out emotional polydypsia without the necessity of a long, carefully supervised period of thirsting and without the discomfort of thirsting when there is true diabetes insipidus present. This is the so-called Hickey-Hare test, which consists in the intravenous infusion of hypertonic saline solution. This ordinarily calls forth an increased secretion of antidiuretic hormone by the posterior pituitary followed by the renal response thereto. Patients with psychogenic polydypsia will respond with a decrease in urine volume, as does the normal. The patient with true diabetes insipidus of

pituitary origin or the patient with renal diabetes insipidus will show no concentration of urine after the infusion of the hypertonic saline solution. These two conditions must then be separated by the response to Pitressin.

TESTS OF FUNCTION OF THE PARATHYROIDS

Diseases of the parathyroid have to be separated from renal disease, and frequently the important procedures actually are the ruling out of renal disease with the usual renal function tests. In addition, the phosphate excretion by the kidneys after the infusion of parathyroid hormone as described by Ellsworth and Howard[3] is the classic test for *hypoparathyroidism* as opposed to *pseudohypoparathyroidism* in the presence of a high phosphorus and a low calcium in the peripheral blood. Balance studies in the response of vitamin D are likewise carried out in parathyroid disease and in pseudohypoparathyroidism.

In following the course of patients with parathyroid disease frequent use is made of the Sulkowitch test, which is a semiquantitative estimate of calcium excretion in the urine which reflects the calcium level of the blood. In the treatment of hypoparathyroidism one usually attempts to maintain the level of calcium so that the Sulkowitch test is positive, but not markedly so.

Howard[9] has recently described a procedure that can be carried out in the routine laboratory. An infusion of calcium salts is given intravenously. In the normal this results in a rise in serum phosphate concentration and a reduction in urinary excretion of phosphate. Hyperparathyroid patients respond with much smaller rises in concentration of serum phosphate and a smaller reduction or an actual increase in urinary phosphate excretion. In hypoparathyroidism the phosphate concentration in the blood does not rise as much as in the normal, and there is a greatly increased urinary phosphate excretion. The percentage of the administered calcium recovered in the urine is higher in the hyperparathyroid patients and lower in the hypoparathyroid patients than in the normal patient.

SUMMARY

In this synoptic report of endocrine function tests the emphasis has been on attempting to point out the value and the deficiencies of the various tests rather than to describe the procedures themselves. The references are an incomplete list of procedures of clinical value in pediatrics; these are in general the ones that can be carried out in the ordinary laboratory.

REFERENCES

1. Barker, S. B.: Determination of Protein Bound Iodine. J. Biol. Chem., *173*:715, 1948.
2. Barney, J. D., and Sulkowitch, H. W.: Progress in the Management of Urinary Calculi. J. Urol., *37*:746, 1937.
3. Ellsworth, R., and Howard, J. E.: Studies on the Physiology of the Parathyroid Glands. VII. Some Responses of Normal Human Kidneys and Blood to Intravenous Parathyroid Extract. Bull. Johns Hopkins Hosp., *55*:296, 1934.
4. Glenn, E. M., and Nelson, D. H.: Chemical Method for the Determination of 17-Hydroxycorticosteroids and 17-Ketosteroids in Urine Following Hydrolysis with Beta Glucuronidase. J. Clin. Endocrinol. & Metab., *13*:811, 1953.
5. Goldenberg, M., Serlin, I., Edwards, T., and Rapport, M. M.: Chemical Screening Methods for the Diagnosis of Pheochromocytoma. Am. J. Med., *16*:310, 1954.
6. Gornall, A. G., and MacDonald, M. P.: Quantitative Determinations of the Steroid Hormones with 2, 4 Dinitrophenylhydrazine. J. Biol. Chem., *201*: 279, 1953.
7. Hawk, P. B., Oser, B. L., and Summerson, W. H.: Practical Physiological Chemistry. Philadelphia, Blakiston Company, 1949, p. 700.
8. Hickey, R. C., and Hare, K.: The Renal Excretion of Chloride and Water in Diabetes Insipidus. J. Clin. Investigation, *23*:768, 1944.
9. Howard, J. E., Hopkins, T. R., and Connor, T. B.: On Certain Physiologic Responses to Intravenous Injection of Calcium Salts into Normal, Hyperparathyroid and Hypoparathyroid Persons. J. Clin. Endocrinol. & Metab., *13*:1, 1953.
10. Jailer, J. W.: A Fluorometric Method for the Clinical Determination of Estrone and Estradiol. J. Clin. Endocrinol., *8*:564, 1948.
11. Klinefelter, H. F., Jr., Albright, F., and Griswold, G. C.: Experience with a Quantitative Test for Normal or Decreased Amounts of Follicle-Stimulating Hormone in the Urine in Endocrinological Diagnosis. J. Clin. Endocrinol., *3*:529, 1943.
12. Nelson, D. H., and Samuels, L. T.: A Method for the Determination of 17-Hydroxycorticosteroids in Blood; 17-Hydroxycorticosterone in the Peripheral Circulation. J. Clin. Endocrinol. & Metab., *12*:519, 1952.
13. Reddy, W. J., Jenkins, D., and Thorn, G. W.: Estimation of 17-Hydroxycorticoids in Urine. Metabolism, *1*:511, 1952.
14. Robinson, F. J., Power, M. H., and Keppler, E. J.: Two New Procedures to Assist in the Recognition and Exclusion of Addison's Disease. Proc. Staff Meet., Mayo Clin., *16*:577, 1941.
15. Shorr, E.: A New Technique for Staining Vaginal Smears. III. A Single Stain. Science, *94*:545, 1941.
16. Talbot, N. B., Sobel, E. H., McArthur, J. W., and Crawford, J. D.: Functional Endocrinology from Birth through Adolescence. Cambridge, Harvard University Press, 1952.
17. Taurog, A., and Chaikoff, I. L.: The Nature of the Circulating Thyroid Hormone. J. Biol. Chem., *176*:639, 1948.
18. Werner, S. C., Hamilton, H. B., Leifer, E., and Goodwin, L. D.: An Appraisal of the Radioiodine Tracer Technic as a Clinical Procedure in the Diagnosis of Thyroid Disorders. Uptake Measurement Directly over the Gland and a Note on the Use of Thyrotropin (T.S.H.). J. Clin. Endocrinol., *10*:1054, 1950.
19. Wilkins, L.: The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence. Springfield, Ill., Charles C Thomas, 1950.

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EXAMINATION OF CEREBROSPINAL FLUID

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Since diseases of the central nervous system may develop without localizing signs and symptoms, frequently the physician must examine the cerebrospinal fluid to establish a diagnosis. Further, because early specific therapy can prevent tragic sequelae in purulent meningitis, it behooves the physician to make a careful, accurate and complete observation of the cerebrospinal fluid. This requires proper methods of collecting and handling of the fluid to assure that the maximum information be obtained. Since much depends upon the immediate examination of the fluid, the physician himself should be able to perform the studies and interpret the results.

MECHANISM OF FORMATION AND CIRCULATION

The elaboration of cerebrospinal fluid has long been thought to be a function of the choroid plexus of the cerebral ventricles, and it was also thought that the fluid flowed from the ventricles into the subarachnoid space to be reabsorbed largely by the arachnoid villi. Through isotope tracer studies in man, Sweet and Lockeley[8] have suggested that the cerebrospinal fluid is formed as extracellular fluid in that water and electrolytes enter both in the ventricles and throughout the subarachnoid space. Thus the formation of the fluid would not be dependent wholly upon the choroid plexus. These studies further indicate that each constituent of cerebrospinal fluid is exchanged with blood at its own characteristic rate and will therefore reflect systemic changes in the individual. Protein, however, is largely reabsorbed by the arachnoidal villi, which serve much as lymphatics do in the general circulation.

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Gardner[2] and others suggest that increase in the cerebrospinal fluid protein molecules associated with brain tumors and other diseases accumulates against the semipermeable membrane of the arachnoid villi and that this prevents reabsorption of fluid and protein. Such obstruction produces an increase in the cerebrospinal fluid pressure.

Regardless of the mechanism for the elaboration and reabsorption of cerebrospinal fluid, the effect of local and systemic disease on the fluid is characteristic enough to be diagnostic. The response to purulent infection of the meninges is a profuse leukocytic exudation in the presence of bacteria. A viral invasion is manifested by a limited lymphocytic response, and bleeding is reflected by the presence of blood in the fluid. Abnormal cells from infection or tumor produce elevation of the protein content of the cerebrospinal fluid, and many metabolic diseases are reflected in the cerebrospinal fluid by changes comparable to that seen in the blood, such as hypocalcemia in tetany. In general, the cerebrospinal fluid behaves as other extracellular compartments, and the specific characteristics of some diseases can be delineated there as elsewhere.

The amount of cerebrospinal fluid in the infant varies inversely with the body size. The brain develops in a fluid-filled space, but at birth the brain nearly fills the skull. Some 10 to 15 cc. of cerebrospinal fluid can be obtained at autopsy of infants weighing 1500 to 2000 gm. if they have been delivered from below. If they are delivered by cesarean section, Potter[6] states that 30 to 60 cc. of fluid can be collected at autopsy. She suggests that delivery through the vagina has forced some of the fluid out of the meninges. The rate of formation of cerebrospinal fluid is unknown, but for practical purposes 1 to 2 cc. may be removed from a premature and 10 cc. from a 10-kilogram infant with no deleterious effects.

The cerebrospinal fluid tension is an effective counterpressure, for it protects the brain from large changes in intravascular pressure. The cerebrospinal fluid pressure varies directly with the venous pressure. This relationship can be demonstrated by determining the cerebrospinal fluid pressure above and below the level of the heart, and the dynamics can be further explored by the Queckenstedt test. This study consists in increasing the venous pressure by gentle compression of the jugular veins. Unless some obstruction is present, the cerebrospinal fluid pressure promptly rises and then falls as the venous compression is released. Such a study should not be done in the presence of increased intracranial pressure. Exact measurement of pressure in the infant and child may be difficult because of the lack of cooperation of the child and the amount of fluid needed to fill the manometer. However, the clinician can estimate roughly the presence of increased pressure by the bulging fontanel and the pressure with which fluid is forced through the needle.

METHODS OF OBTAINING CEREBROSPINAL FLUID

Lumbar Puncture

The simplest method of obtaining fluid is by lumbar puncture if there is no evidence of prolonged increase of intracranial pressure or evidence of choked disks. This is most easily done with the infant flexed and lying on his side. With sterile precautions the needle is introduced between the second and third lumbar vertebrae. It is useful to permit about eight drops of fluid to drop directly on a chocolate agar slant for culture. Two other sterile tubes are used to collect 5 additional cc. This is sufficient to use one for cell counts and chemistry and the other for bacteriologic studies. Manometric studies can be performed if indicated on the older children.

Cisternal Tap

Occasionally because of obstruction, increased intracranial pressure or inability to obtain cerebrospinal fluid for other reasons, a cisternal tap is

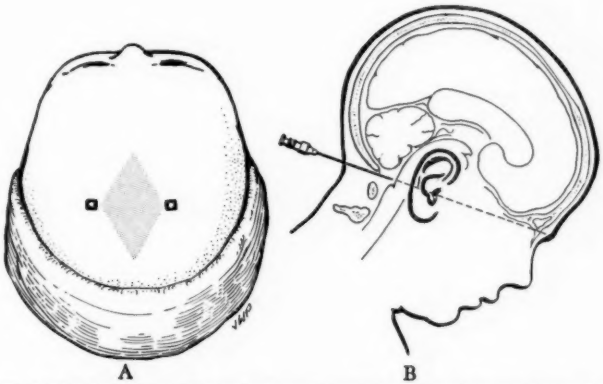

Fig. 27. A, Subdural taps. The spinal needles are introduced at a right angle to the skin in a suture line just outside the angle of the fontanel. Note that the head is shaved. B, Cisternal tap. The needle is passed in the midline below the occiput and in a line extending through the external auditory canal and the glabella.

necessary. The occiput and neck are first shaved, and the usual sterile precautions are taken. Then, with the head sharply flexed and the child in a sitting or lying position, the needle is introduced through the skin in the midline at the base of the occiput. The needle is then directed in a line from the base of the occiput through the external auditory meatus to the glabella. The cisterna lies at a depth of 1 to 2 cm. in infants[1] and

4 to 4.5 cm.[7] in the adult. Care must be taken not to exceed these depths. The needle is introduced cautiously, removing the stilet from time to time, until the cisterna is entered. The fluid is collected as outlined for cerebrospinal fluid.

Subdural Tap

This method is discussed in another clinic in this volume (p. 239) and is mentioned here only for completeness.

ROUTINE EXAMINATION OF CEREBROSPINAL FLUID

The following routine examination should be performed on all cerebrospinal fluid:

Appearance. Upon examination in daylight the fluid should be clear and colorless. Turbidity and xanthochromia are abnormal.

Pressure. Normal pressure is 40 to 110 mm. of water in infants, and up to 200 mm. in adults. This rises and falls promptly with gentle jugular compression.

Cell Count. The cell count may be obtained by placing the cerebrospinal fluid in a hemacytometer counting chamber. All nine squares are counted, and the total number of cells is multiplied by 10/9. To lyse the red cells and stain the white cells the fluid can be diluted 1:10 in a white cell pipet with a staining fluid of glacial acetic acid and crystal violet. The normal cell count is 0 to 10, and these cells are mononuclear. If blood is present, owing to a bloody tap or cerebrovascular hemorrhage, the total cell count should be first obtained without dilution with the acetic acid. Then the fluid can be diluted and recounted. If more than 10 cells are present, a differential count of the centrifuged sediment can be done by using Wright's stain.

Protein. The protein content can be roughly estimated by the Pandy test or by a number of other tests. The Pandy test consists in precipitation of protein in a saturated solution of phenol. It is wise to warm the Pandy solution first if it is cloudy. A drop of cerebrospinal fluid is then introduced into a test tube containing about 2 cc. of Pandy solution. Normally, a faint trace of precipitate may be seen after the descent of the crop. If the protein value is high, the Pandy solution will be grossly cloudy. If blood is present, the Pandy test will be positive. The quantitative determination varies with the individual laboratory, and the normal values range between 15 and 45 mg. per 100 cc. If high protein content exists, a pedicle will form in the cerebrospinal fluid upon standing.

Sugar. The sugar determination may be roughly estimated by using five test tubes containing 1 cc. of Benedict's solution and introducing

one, two, three, four and five drops of cerebrospinal fluid into individual tubes. These are then placed in a bath of boiling water for five minutes. Reduction is evident in the fourth and fifth tubes only if the sugar content is normal. If the sugar is low as in meningitis, no reduction is seen. On the contrary, diabetes may be suspected because of 4+ reduction in all the tubes. The quantitative sugar values vary between 40 and 80 mg. per 100 cc., depending upon the blood sugar, which is usually about 50 per cent higher.

Chloride. The normal values are 116 to 125 mg. per liter (675 to 730 mg. of sodium chloride per 100 ml.).

Bacteriology. All cerebrospinal fluid should be examined for organisms if meningitis is suspected even though the fluid appears clear. The centrifuged sediment is smeared, stained and examined. In addition, culture of the cerebrospinal fluid should be a routine procedure. It has been my experience that if about eight drops of fluid are dropped directly on a chocolate agar slant and then incubated, the number of positive cultures is significantly higher than when culture materials are subsequently inoculated with cerebrospinal fluid in the laboratory.

Other. Other special studies such as Wassermann, colloidal gold and cell blocks are done as indicated.

CEREBROSPINAL FLUID IN THE NEWBORN

The fluid in the newborn is frequently xanthochromic, is under less pressure and is less in amount than in the older infant. Because of the size of the infant, and the low volume and pressure, the lumbar puncture is technically more difficult than in the older infant. I have followed the suggestion of Glaser,[3] who suggests using a small needle (no. 24 or 26) and placing the infant in a sitting position. Presumably this position distends the lumbar sac and permits easier entry. By attaching a syringe to the needle 1.5 to 2 cc. may be withdrawn. This fluid is frequently xanthochromic and contains both red and white blood cells. Since the traumatic tap is not uncommon in the newborn and premature, the significance of the blood is not always clear. If the cerebrospinal fluid tends to become clearer as it is obtained, if the cells are not crenated, and if after centrifugation the supernatant fluid is only slightly xanthochromic, this suggests that the blood is traumatic in origin. This is not absolute, and the evaluation of the findings rests upon the clinical judgment of the operator. The protein in the cerebrospinal fluid is higher (60 to 90 mg. per 100 cc.) than in older infants, but with cerebral hemorrhage it is frequently 300 to 500 mg. or more. The cerebrospinal fluid sugar and chloride are normal.

Because of the technical difficulties the value of a lumbar puncture in the newborn is questioned. Certainly if neurologic symptoms are

present, it seems indicated, since clinically one cannot distinguish meningitis from cerebral hemorrhage. Even though cerebral damage and hemorrhage may not be evident upon cerebrospinal fluid examination, an attempt at evaluation should be made. There is little evidence that removal of red cells by repeated cerebrospinal fluid taps is therapeutically valuable.

CEREBROSPINAL FLUID IN DISEASE

nfection of Central Nervous System

Purulent Meningitis. MENINGOCOCCUS, HEMOPHILUS INFLUENZAE AND PNEUMOCOCCUS. Examination of the cerebrospinal fluid in purulent meningitis is imperative, since the therapy can be tailored to the specific infection. The fluid is under increased pressure as evidenced by the bulging fontanel as well as the actual manometric measurement. The fluid is usually turbid, but the appearance can be deceiving and a cell count should be done. The cell count is usually grossly elevated to 5000 to 10,000 cells per cubic milliliter, predominantly polymorphonuclear leukocytes. However, I have seen both a positive smear for pneumococci and a positive culture for meningococci in the presence of a normal cell count. A gram stain of centrifuged sediment may show the organism. However, sterile precaution must be taken, since the fluid becomes contaminated easily. The common organisms found in children are meningococcus, *H. influenzae* and pneumococcus. The last is easily found, but a search usually must be made for the former two. Because of the frequent use of antibiotics early in the disease before a diagnosis has been established, the organisms may be difficult to find. For this reason the culture should be carefully examined twelve and twenty-four hours later to confirm or establish the diagnosis. The *H. influenzae* can be confirmed from the smear or culture by the Quellung reaction with specific antiserum. If the meningococcus is suspected but not found, some laboratories are equipped to do type-specific antiserum titers on the cerebrospinal fluid. This is not yet generally available.

Sugar in purulent meningitis is frequently low (0 to 10 mg. per 100 ml.), depending on the severity of the disease, and may be sometimes useful as a guide to prognosis. However, a normal value for the cerebrospinal fluid sugar is not infrequent even in fulminating meningitis. Rather, it is useful if a gradual rise is demonstrable associated with therapy. The protein is normal or slightly elevated initially and gradually rises during the first forty-eight to seventy-two hours, falling to normal on convalescence. The chloride is normal.

ESCHERICHIA COLI AND OTHERS. Occasionally newborn infants have

meningitis due to *Escherichia coli, Staphylococcus albus* or other less virulent organisms. The cerebrospinal fluid response is essentially the same, but the disease is less severe. There is increase in pressure, cells and protein, and the sugar is slightly depressed. The organisms are recovered by smear and culture. The disease is less fulminating, however, and the cerebrospinal fluid changes are less dramatic. It is always useful to examine carefully for source of infections such as an intraspinal or intracranial dermoid cyst in the presence of an indolent meningitis, since this may be the source of the infection.

TUBERCULOSIS. The meningeal reaction to tuberculosis differs somewhat from other bacterial infection. The cell count is never as high, usually 50 to 400 cells with about 70 per cent lymphocytes. The fluid is, therefore, clear to opalescent. The protein is elevated and the sugar characteristically low. This fluid usually demonstrates low chloride content (85 mg. per liter), and this can be of diagnostic importance. The tubercle bacillus may be found on smear with an acid-fast stain, but more success is obtained by culture in guinea pig on an artificial medium such as that of DuBos.

ENCEPHALITIS. Involvement of the central nervous system by a specific virus is not characteristic, and differentiation frequently must be made on other clinical and serologic grounds. However, evidence for a viral encephalitis consists of clear fluid under slightly increased pressure with moderate increase in cells in the neighborhood of 150 to 250 per cubic milliliter. Most of these cells are lymphocytes. There is usually a moderate increase in the protein, but the sugar and chloride determinations are normal. However, in spite of clinical findings of encephalitis the cerebrospinal fluid may be normal. Under these circumstances immunologic studies for mumps, herpes, Eastern and Western equine and St. Louis encephalitis must be done. Lymphocytic choriomeningitis is suggested if the cerebrospinal fluid cell count is around 1000 per cubic milliliter or more and the smear reveals that all cells are lymphocytes. This diagnosis can and should be confirmed by serologic studies.

Poliomyelitis produces an unusual response in that, if the studies are done early in the disease, the polymorphonuclear leukocyte predominates. This is reversed within forty-eight to seventy-two hours after onset. Otherwise the findings are those of any viral infection. Tissue culture of the poliomyelitis virus is still in the experimental stage. A positive stool culture for the Coxsackie virus does not eliminate poliomyelitis, since both infections may exist concurrently.

Parasitic Diseases

Infection by *Torula histolytica* most frequently gives signs of central nervous system involvement. The cerebrospinal fluid appears opalescent,

TABLE 13. *Cerebrospinal Fluid Values*

TYPE	APPEARANCE	PRESSURE	CELL COUNT	PROTEIN	SUGAR	CHLORIDE	REMARKS
Normal	Clear	40–110 mm./ H_2O	0–8 lymphocytes	15–45 mg.%	60 mg.%	116–125 mg./L.	
Bloody tap	Bloody	Normal	Numerous RBCs	15–45 mg.%	Normal	Normal	RBCs not crenated; supernatant fluid only slightly xanthochromic
Newborn	Xanthochromic	Low	Few RBCs	50–90 mg.	Normal	Normal	Few crenated RBCs; supernatant fluids xanthochromic
Cerebrovascular hemorrhage	Bloody	Normal to high	Numerous RBCs	100 mg.%	Normal	Normal	Many crenated RBCs; supernatant fluid xanthochromic
Purulent meningitis	Cloudy	Elevated	Numerous WBCs, predominantly polymorphonuclears	Elevated 50–100 mg. %	Low	Normal	Gram stain may reveal organisms; early bacteriology may be positive before evidence of cellular response
Tuberculous meningitis	Opalescent	Slight. elev.	100–500 cells 70% lymphocytes	Elevated	Low	Low	Smear and culture are positive; fluid may form pedicle on standing

Viral encephalitis	Clear to opalescent	Normal to slight. elev.	0–500 cells 90% lymphocytes	Slightly elevated	Normal	Normal	Frequently completely normal; serologic differentiation
Poliomyelitis	Clear to opalescent	Elevated	50–500 cells 70% lymphocytes	Slightly elevated	Normal	Normal	Early the cell count is predominantly polymorphonuclear cells
Lymphocytic choriomeningitis	Opalescent	Slight. elev.	1000 cells Lymphocytes	Elevated	Normal	Normal	Serologic studies positive
Guillain-Barré syndrome	Clear	Elevated	Normal	Elevated	Normal	Normal	
Meningismus	Clear	Slight. elev.	Normal	Normal	Normal	Normal	Clinical evidence of meningeal irritation only
Lead encephalitis	Clear	Elevated	8–50 cells	Elevated	Normal	Normal	
Tumor and abscess	Clear	Elevated	8–300 cells	Elevated	Normal	Normal	May be normal
Subdural hematoma in infants	Clear to xanthochromic	May be elevated	May have RBCs	Normal to elevated	Normal	Normal	CSF may be normal, but xanthochromic fluid in subdural taps with high protein content

the cell count and protein are increased, and the diagnosis may be established by demonstrating budding yeast cells on smear or in culture.

Space-Occupying Lesions

The examination of the cerebrospinal fluid in the presence of a tumor, abscess or hematoma can be dangerous, yet helpful in diagnosis. In the presence of increased intracranial pressure the danger lies in removal of fluid from below the lesion, permitting the cerebellar tonsils to herniate through the foramen magnum. This accident may occur with the rapid removal of fluid even in purulent meningitis when a partial block is present due to exudate at the base of the brain. It is, therefore, wise to obtain fluid from the cisterna or ventricle if such a condition is suspected. Under these circumstances the fluid is under increased pressure, and a moderate increase in mononuclear cells may be present, but the protein is usually significantly increased. The remaining constituents are normal. This does not differentiate the lesion, but does suggest the presence of one.

Systemic Diseases

Infections localized outside the central nervous system may produce signs of meningeal irritation without producing significant change in the cerebrospinal fluid examination other than a slight increase in pressure. This is designated *meningismus* and frequently accompanies upper and lower respiratory infections, but may occur with infections at any site. *Acute plumbism* produces signs of meningeal irritation and convulsions. The cerebrospinal fluid examination shows an increase in pressure, and elevated protein, but is otherwise normal. Examination in *polyradiculitis (Guillain-Barré syndrome)* produces protein level of 100 to 200 mg. per 100 cc. in the presence of normal cell count and evidence of increased cerebrospinal pressure. Diagnosis of *diabetes* is not infrequently established in an infant with central nervous system lesions because of a high cerebrospinal fluid sugar. Hallman and Tähkä[4] have demonstrated that *diarrheal diseases* are frequently accompanied by an increase in lymphocytes (50 to 100 per cubic milliliter) and elevated protein in the cerebrospinal fluid. If *salicylism* is suspected, the fluid may be tested for salicylate with ferric chloride. This test consists in adding 2 or 3 drops of 10 per cent chloride to the cerebrospinal fluid. A dark purple color indicates the presence of salicylates. This is positive only if the blood levels have reached toxic concentration. Although distinctly a less common problem, congenital and juvenile *syphilis* are reflected in the cerebrospinal fluid by positive Wassermann tests. During the acute

syphilitic meningitis the cells and protein are elevated, and in juvenile syphilis the paretic gold curve may be obtained.

SUMMARY

The examination of the cerebrospinal fluid is an important diagnostic procedure if the fluid is carefully studied and interpreted. The characteristic responses are outlined in Table 13. In general, inflammatory and metabolic diseases are reflected in the cerebrospinal fluid examination as they are in other extracellular fluid spaces in the body. With careful examination of the fluid, and cautious interpretation in the clinical situation, there is good reason to make an exact diagnosis so that specific therapy can be used.

REFERENCES

1. Ford, F. R.: Diseases of the Nervous System in Infancy, Childhood and Adolescence. 3rd ed. Springfield, Ill., Charles C Thomas, 1952.
2. Gardner, J. W., Spitler, D. K., and Whitten, C.: Increased Intracranial Pressure Caused by Increased Protein Content in the Cerebrospinal Fluid. New England J. Med., 250:932, 1954.
3. Glaser, J.: The Cerebrospinal Fluid of Premature Infants. Am. J. Dis. Child., 36: 195, 1928.
4. Hallman, N., and Tähkä, H.: Observations on the Cerebrospinal Fluid in Infantile Diarrhea. Acta paediat., 41:437, 1952.
5. Potter, E. L.: Pathology of the Fetus and the Newborn. Chicago, Year Book Publishers, Inc., 1952.
6. Santamarina, V.: La punction sub-occipital o cinsernal en la clinica infantil. Arch. de la Soc. de Estud. Clin. de la Habana: 32:57, 1938. Cited in Ford, F. R.[1]
7. Sweet, W. H., and Locksley, H. B.: Formation, Flow and Reabsorption of Cerebrospinal Fluid in Man. Proc. Soc. Exper. Biol. & Med., 84:397, 1953.

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SUBDURAL TAPS

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The dura mater is a tough, fibrous membrane, adherent to the inner surface of the calvarium; the pia-arachnoid is a filmy, transparent structure closely attached to the brain. The compartment between the dura and the pia-arachnoid is a continuous slitlike space which under normal circumstances contains a few drops of fluid and permits a certain amount of movement of the brain within the intracranial chamber. This subdural space is traversed by a number of bridging veins which carry blood from the cortical circulation to the large venous channels within the dura.

INDICATIONS

In infancy, fluid accumulates in the subdural space to a pathologic degree principally under one of two conditions:

1. As a result of cranial moulding or a sudden deceleration type of *injury*, such as when the moving head strikes a solid object, the veins bridging the subdural space are stretched or torn with resultant venous hemorrhage. This may result in formation of a solid hematoma or, more often, in a collection of bloody fluid of high protein content which subsequently becomes encapsulated in a membranous envelope.[1, 2]

2. Subdural effusion also occurs in infancy as an inflammatory response to bacterial *infection* in the adjacent pia-arachnoid. The amount and character of fluid found in the subdural space after meningitis vary widely, depending on a number of factors: the type of organism causing the meningitis, the promptness and efficacy of antibiotic treatment, and the time after onset of meningitis when subdural tap is first performed.[3, 4]

The indications for puncture of the subdural space, both in traumatic and infectious conditions, concern both diagnosis and treatment. In fact, the initial positive tap which confirms the diagnosis of subdural

hematoma or postmeningitic subdural effusion also marks the beginning of effective therapy. A positive tap is in itself indication for further taps, until the subdural spaces are dry or until more definitive therapy has been carried out.

The principal indication for carrying out initial subdural tap in subdural hematoma is simply suspicion of the possibility of this lesion. There is no characteristic clinical syndrome. In an infant during the first few months of life there may be nothing more than failure to gain weight, refusal of feedings, hyperirritability, and perhaps slightly accelerated rate of head growth. Convulsions, vomiting, low grade fever, bulging of the anterior fontanel are frequent but by no means constant features.

After meningitis, in the first year of life particularly, the indication for initial subdural taps is failure to respond quickly and satisfactorily to adequate antibiotic treatment. More specifically, such exploratory subdural taps should be performed on any infant with meningitis who shows any of the following: (1) fever after forty-eight to seventy-two hours of adequate specific and supportive treatment; (2) persistent positive cerebrospinal fluid cultures; (3) focal or persistent convulsions; (4) persistent vomiting; or (5) focal neurologic disturbances after subsidence of infection.

EQUIPMENT

The equipment necessary to carry out diagnostic or therapeutic subdural taps is not elaborate and is generally available. It consists of the following:

1. Sterile rubber gloves and a sterile towel
2. Skin antiseptic solutions, such as alcohol and aqueous Zephiran (iodine is not recommended for repeated use on the infant's scalp)
3. Short-beveled, sharp, 1½- or 2-inch lumbar puncture type needles with accurately fitting stilets, size number 19 or 20
4. 0.5 to 1 per cent procaine, with hypodermic needle and syringe
5. Sterile collecting test tubes
6. Rubber nipple stuffed with cotton saturated with glucose solution for additional analgesia is desirable.

TECHNIQUE

Tapping the subdural space is an extremely simple procedure which is readily carried out in the office or hospital treatment room and is entirely safe, provided the strictest asepsis is observed and a fairly exact routine is followed. Since the early diagnosis of subdural hematoma and of subdural effusion complicating meningitis can be made by subdural tap and by this means only, it is important that pediatricians learn to perform this procedure with ease and accuracy.

Subdural tap is sometimes loosely referred to as "fontanel puncture." This term is mentioned only to disparage it, since taps should be carried out instead through the coronal suture well lateral to the anterior fontanel. It is a safe rule to stay always at least 1½ inches from the midline, or at a point approximately in line with the pupil of the eye.

The infant should be securely restrained by wrapping it in a sheet or blanket in such a fashion as to immobilize the arms and legs completely. The baby is then held on a treatment table or across a crib so that the whole top of the head is accessible. If a nurse or assistant holds one hand on either side of the head and the baby is given a sugar nipple as pacifier, there is usually no difficulty controlling resistance. The scalp should be prepared as carefully as for any surgical procedure. After shaving a wide area (at least all hair anterior to a line between the ears), suitable skin antiseptics are used. It is unwise to use excessive draping of the head because of the desirability of keeping all landmarks under vision. One sterile towel covering the posterior part of the vertex only is recommended. The operator should scrub his hands thoroughly and use sterile rubber gloves.

The coronal suture is identified and a procaine wheal placed in the overlying skin for anesthesia. The short-beveled, 2-inch lumbar puncture needle is then introduced through the scalp at a right angle to the skin surface. The needle is pushed in with the right hand while the left hand rests against the head in such a way that the shaft of the needle is grasped between the thumb and forefinger. In this manner lateral deviation of the point can be controlled satisfactorily even if the infant moves its head a good deal. With the point of the needle through the scalp, it is moved slowly along the bone until the suture line is encountered. The needle is then pushed ahead steadily and slowly until a feeling of resistance is suddenly overcome—indication that the subdural space has been entered. In older infants, when there is firm fibrous union of the suture, it may be necessary to "screw" the needle through until the dura is penetrated.

If there is a pathologic amount of subdural fluid, it is always encountered immediately beneath the dura, so that there is no need to insert an exploring needle further once the dura has been punctured. The stilet is removed and fluid allowed to drip from the needle. It may take a moment or two for good flow to be established. The needle can be rotated to alter position of the bevel, but the point should not be moved sideways. Attachment of a syringe for aspiration, or attempted aspiration, of fluid from the subdural space is contraindicated. After 15 to 25 cc. of fluid have been removed the needle is withdrawn and digital pressure exerted through sterile gauze at the puncture site for a moment or two; a small dry sterile dressing is then applied.

In this Clinic it is customary to perform bilateral taps always for diagnosis. If both are positive, from 10 to 20 cc. of fluid are removed from each side. When subsequent therapeutic taps are performed, it is customary to tap one side each day alternately, removing 10 to 25 cc. of fluid each time. It is felt unwise to remove more than this, particularly in very sick children with increased intracranial pressure, since the sudden marked intracranial pressure change incident to removal of larger amounts has occasionally proved disastrous. If removal of 25 cc. of subdural fluid once a day does not relieve symptoms of increased pressure or keep the anterior fontanel from becoming tense, then taps are simply repeated at more frequent intervals, still with removal of no more than 25 cc. each time.

When punctures are continued for several days, the point of needle

insertion should be varied along the coronal suture line slightly, since repeated entry through the same needle hole is apt to produce a fistula with persistent drainage.

At the first taps, cultures should be made as well as cell counts and total protein determinations, unless the fluid is grossly bloody. It is useful to keep a sample of fluid from each tap in a test tube to compare color changes from day to day. In following the character of post-meningitic subdural effusions, it is wise to obtain culture, cell count, and total protein determination of every sample removed.

DISCUSSION

How long subdural taps should be continued depends upon the character of the fluid obtained and the infant's general condition. In this Clinic it is felt that in every instance when high protein, xanthochromic or bloody fluid is obtained repeatedly from the subdural space in amounts of more than a few cubic centimeters, burr-hole exploration is indicated to establish whether or not a subdural membrane has formed. If a few cubic centimeters only of lightly colored fluid are obtained and this disappears after three or four taps, nothing more is done. This situation is often found after mild trauma and in the course of meningitis which has been treated promptly and adequately.

If subdural taps become quickly negative, but the baby continues to have any symptoms, it is wise to wait a few days and repeat the taps, since occasionally there will be reaccumulation of fluid under these circumstances. If the subdural fluid is grossly bloody and obtained in large amounts, it is wise to continue tapping until there is no further evidence of active bleeding and the baby's general condition has been improved by whole blood transfusions and other supportive therapy before surgical treatment is pursued.

REFERENCES

1. Ingraham, F. D., and Matson, D. D.: Subdural Hematoma in Infancy. J. Pediat., 24:1, 1944.
2. Ingraham, F. D., and Matson, D. D.: Neurosurgery of Infancy and Childhood. Springfield, Ill., Charles C Thomas, 1954.
3. McKay, R. J., Jr., Ingraham, F. D., and Matson, D. D.: Subdural Fluid Complicating Bacterial Meningitis. J.A.M.A., 152:387, 1953.
4. Matson, D. D.: Subdural Effusion Complicating Bacterial Meningitis in Infants. Tr. Am. Neurological A., 1953, p. 123.

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BONE MARROW EXAMINATION IN CHILDREN

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Examination of the bone marrow has become a well established and essential procedure, not only in the study and treatment of hematologic disorders, but in a variety of other pediatric problems as well. Bone marrow biopsy is indicated in the diagnostic problems presented by disorders affecting the peripheral blood picture as may occur in obscure infections; in infiltrative diseases such as malignant lymphoma and neuroblastoma; and in suspected lipoprotein metabolic disorders. Careful and repeated study of the marrow is imperative during therapy with medicinal agents which may depress hematopoiesis, and is essential in the evaluation of therapy in leukemia and certain of the anemias. Its value, then, is unquestioned; however, this value is in direct proportion to the adequacy of the sample. The usual techniques and indications for bone marrow examination have been well summarized by Custer,[3] Limarzi et al.[5] and Smith.[6] The purpose of this paper is to discuss some aspects of the technique of bone marrow biopsy and its application in pediatric practice.

TECHNIQUE OF BONE MARROW ASPIRATION AND BIOPSY IN CHILDREN

Preparation of bone marrow material is not difficult; however, preparation of good marrow smears or spreads requires practice, as evidenced by the frequency with which unsatisfactory slides are submitted for evaluation. Needle aspiration of the marrow usually provides adequate material for study. The spreads, preferably made by the cover-glass

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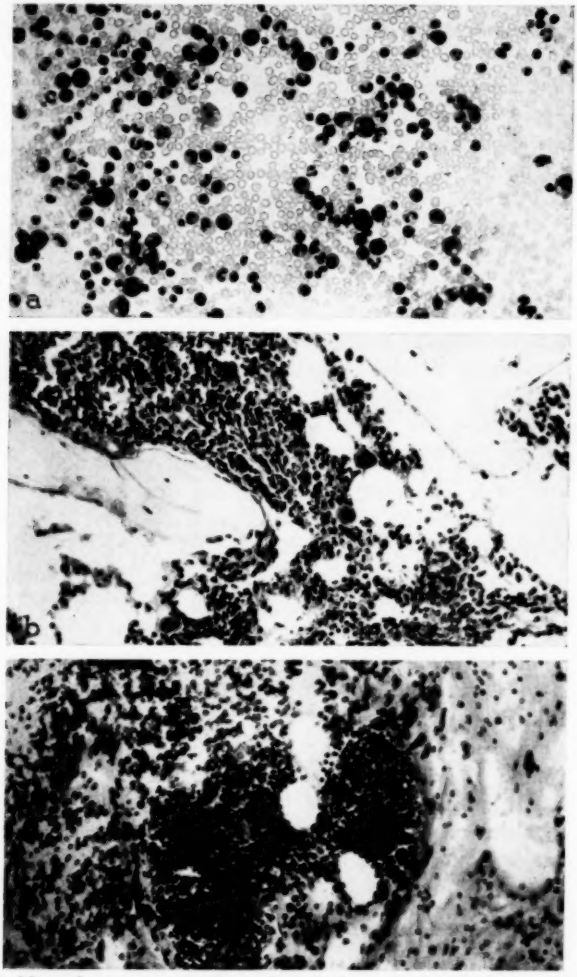

Fig. 28. Normal marrow spread. *a*, Spread shows good cellularity with normal differentiation of cell types. × 300. *b*, Section of bone plug. Specimen shows marrow spaces well filled with hematopoietic tissue and some fat. × 300. *c*, Section of clot. Cells are well differentiated and may be distributed diffusely or in concentrated groups. × 300.

method, are dried in air and stained with either Wright's and Giemsa's or Jenner's and Giemsa's stains. Additional valuable information may be obtained from sections prepared from a clot,* curettings† and

* The simplest technique is to permit 0.5 to 1.0 ml. of marrow aspirate to remain undisturbed in the syringe for three to five minutes until a clot is well formed. The clot is semi-adherent to the flat surface of the plunger and may be removed with it

from a "plug" obtained with a Turkel trephine.[8] The contents of the trephine (bone plug) and the clot are each wrapped in small squares of lens paper, fixed in Zenker's acetic acid and processed as surgical specimens.

Marrow aspiration and biopsy can be accomplished with local anesthesia. We prefer preliminary sedation with pentobarbital followed by nitrous-oxide-oxygen anesthesia administered by an anesthetist. This assures a quiet patient, and adequate sampling of the bone marrow can be done with ease. Sternal biopsies may be done repeatedly in this manner.

Sites other than the sternum are preferable when the child is not quiet and relaxed. In infants less than one year of age the site of choice is the tibia just below the tubercle. The iliac crest also is a desirable site in small children. The vertebral spine, iliac crest and sternum are all desirable sites in children ten years of age or more.

Infection of the operative site can be avoided by sterile surgical technique. Since hemorrhagic tendency is not rare among patients in whom bone marrow biopsy is indicated, there may be vigorous bleeding which usually may be controlled by immediate firm pressure. Packing with absorbable hemostatic material may be necessary or desirable in some instances. Deviation from the midline, with possible hemothorax and mediastinal emphysema, is a hazard in biopsy of the sternum, but with proper experience and care perforation of the posterior sternal table can be avoided.

The additive information resulting from adequate bone plug and marrow clot biopsies will be illustrated with photographs of the diagnostic problems to be discussed. The histology of normal marrow is illustrated in Figure 28 for comparison. Adequate marrow samples may be difficult to obtain at times even by the most experienced operator, but repeated trials at varying sites usually result in preparations satisfactory for diagnostic purposes.

ESSENTIAL INDICATIONS FOR BONE MARROW STUDIES

Anemia

Bone marrow should be examined to establish a correct diagnosis in the presence of any anemia in children not explainable by blood loss, obvious infection or inadequate diet. Biopsy is especially indicated in the presence of a leukopenia, thrombocytopenia or an abnormal differential blood picture in order to exclude the possibility of leukemia.

from the handle end of the syringe. Another technique involves centrifuging 1 to 2 ml. of marrow with heparin and saline. The top layer then may be clotted by treating with a drop of thrombin and a drop of fibrinogen.

† Marrow particles obtained through the Turkel needle with a sharp curet may be added to the heparinized material before clotting. Curetting should be performed under general anesthesia.

Aplastic Anemia. This may be defined as a severe bone marrow suppression which includes diminution of all cellular elements. This condition may be confused with leukemia on the basis of the peripheral blood studies; thus precise differentiation must be made by bone marrow biopsy. Examination of simple aspirations may be misleading, giving the impression of marrow aplasia when it may be sample inadequacy, due either to focal aplasia or to dilution. It is, therefore, important to examine the plug and clot; and even when this is done, the diagnosis of true aplastic anemia often can be made only by repeated sections

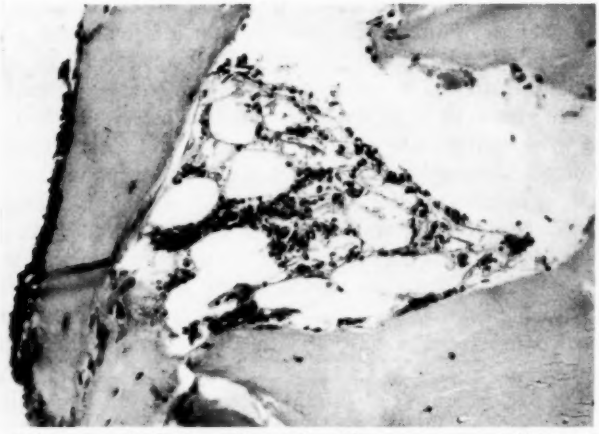

Fig. 29. Aplastic anemia. Section of bone plug. The marrow space is filled with fat and only relatively few cells. × 300. (The clot and marrow spreads showed very few cells.)

from different sites. The following case history illustrates the diagnostic problem.

Case I. A 12 year old girl suffered from frequent upper respiratory infections with little response to antibiotics. In February, 1954, she had coryza, cough, and headache, with fever of 101° F. Her infection did not respond to combined penicillin and sulfonamide therapy, but responded promptly to Chloromycetin therapy.

In April, 1954, the child had another upper respiratory infection, and her mother noted blisters or "bloody patches" on the child's hard palate and "purple" spots on the legs. The child complained of fatigue, and discomfort in her elbows. Her family physician found her hemoglobin to be 10 gm.

On admission the physical examination showed a thin, pale, lethargic girl with a temperature of 104° F. She had moderate cervical lymphadenopathy, numerous petechiae and ecchymoses, but no hepatomegaly or splenomegaly. Peripheral blood findings were: hemoglobin, 9.5 gm.; platelets, 22,000 per cubic milliliter; leukocytes, 900 per cubic milliliter. The differential count showed 6 per cent band forms, 91 per cent lymphocytes, 1 per cent monocytes and 2 per cent young fragmented cells. Examination of the bone marrow confirmed the diagnosis of aplasia by the presence of small numbers of all cell types and replacement of marrow with large quantities of fat (Fig. 29).

Hypoplastic Anemia or Aregenerative Anemia. This means hypoplasia of the red cell series in the marrow, usually without abnormality in the other cellular elements. Differential diagnosis with respect to aplastic anemia is important, since the platelets and leukocytes usually are adequate in hypoplastic anemia. The hazards of thrombopenia with purpura and the repeated infections consequent to the leukopenia characteristic of aplastic anemia are rarely problems.[7] Here, too, marrow biopsy, in addition to aspiration, may be helpful.

Megaloblastic Anemia. This should be considered when macrocytosis is noted in the peripheral blood preparation and is associated with an elevated mean corpuscular volume. A definitive diagnosis may be made only when megaloblasts are demonstrated in the bone marrow. Megaloblastic anemia may be of two types: (*a*) the nutritional anemia of infancy as first described by Zuelzer,[9] which is probably secondary to folic acid deficiency, and (*b*) the true pernicious anemia of children, which is rare. Marrow aspiration alone is sufficient for diagnosis.

Acute Leukemia

Acute leukemia may be defined as a replacement of bone marrow to varying degrees with stem or blast cells. The peripheral white blood

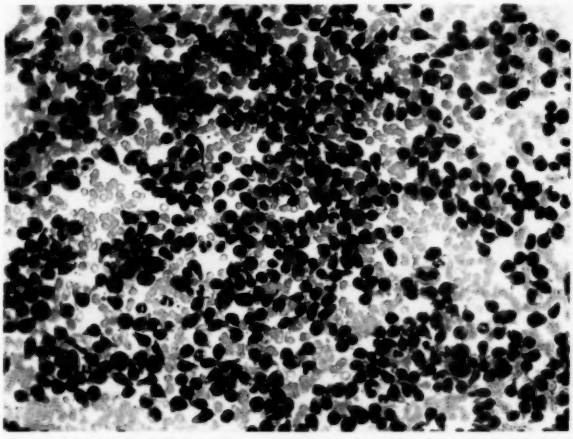

Fig. 30. Acute leukemia. Marrow spread. Predominant cell is the "blast" with relatively few cells of the normal series. × 300. (Plug and clot presented a similar picture of marrow replacement by leukemic cells.)

cell count may be normal, low or elevated. Differential counts may show no blast forms. It is essential to examine the marrow in patients with leukopenia in order to differentiate acute leukemia from aplastic or an occasional atypical hypoplastic anemia, or other diseases which

present disturbances in the peripheral blood picture, particularly those of infectious origin. In addition to peripheral blood changes, physical signs and symptoms initially may suggest such diagnoses as osteomyelitis, "collagen" disease or infectious mononucleosis. Marrow studies are the best means of establishing the correct diagnosis. The aspirate frequently presents a uniformity of cell type, and the diagnosis of acute leukemia is obvious. If the abnormal cells are of sufficient maturity, the type of leukemia may be identified, but in many instances the abnormal cells are undifferentiated and can be classified only as stem cells.

That acute leukemia may clinically suggest rheumatic fever is illustrated in the following history.

CASE II. A 15 year old girl was apparently well until September, 1951, when she complained of pain in her right arm and thigh. She was admitted to a hospital where findings of pain in the right knee, grade I systolic murmur, and temperature of 101° F. suggested rheumatic fever. Hemoglobin of 8.1 gm. and persistent leukopenia throughout her stay remained unexplained. The differential count on October 23, 1951, was considered normal. The results of throat cultures were noncontributory. The child had abdominal pain from time to time and frequently had a slight fever. She had no hepatomegaly, splenomegaly or lymphadenopathy. Bone marrow examination on December 1, 1951, revealed 84.8 per cent blasts (Fig. 30), and a diagnosis of acute leukemia was made.

Chronic Leukemia

Chronic leukemia is a rare pediatric problem representing about 1 per cent of the incidence of leukemia in children and is usually myelogenous in type. The leukocyte count usually is elevated to a considerable degree, and the differential count shows a large number of immature as well as mature granulocytic cells and rare blast cells. The platelet count may be elevated, and anemia usually is present. Bone marrow studies present much the same picture with respect to the granulocytic cell series as in the peripheral blood. Erythrocytic cells are markedly decreased, and megakaryocytes commonly are numerous.

Chronic myelogenous leukemia may eventually "convert" to a typical hematologic picture of acute leukemia as the disease progresses. Repeated bone marrow studies may be the only means of noting this change early in the course of the disease. Chronic lymphatic leukemia in children is extremely rare.

Bone Marrow Metastasis

Although diagnosis of solid tumors usually is established by history, physical examination, x-ray studies and tissue biopsy, bone marrow biopsy has facilitated the diagnosis in many instances when the results of other diagnostic procedures were not confirmatory. In addition, the presence of metastases in the bone marrow indicates widespread dis-

semination and affords some prognostic value. A malignant lymphoma, initially localized in the lymph nodes, may eventually involve the bone marrow and present a picture of acute leukemia. Although Hodgkin's disease does not present a characteristic bone marrow picture, an eosinophilia and the presence of reticulum cells may lead to such a

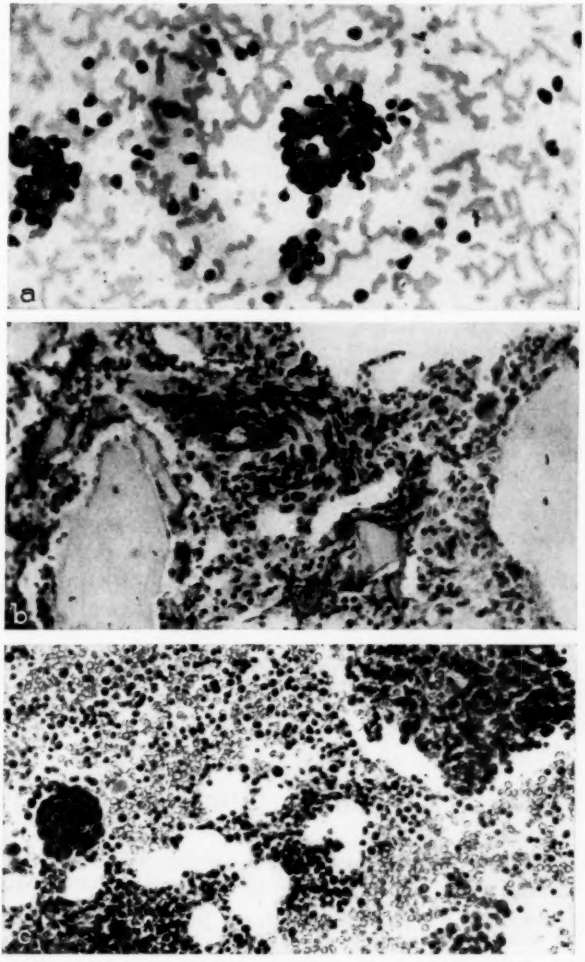

Fig. 31. Neuroblastoma. *a*, Marrow spread. A clump of tumor cells strongly suggests "pseudorosette" formation more clearly demonstrated in tumor biopsy sections. Single tumor cells are scattered throughout. × 300. *b*, Section of bone plug. An aggregate of tumor cells is surrounded by normal marrow. × 300. *c*, Section of clot. Clumps of tumor cells. × 300.

high index of suspicion that biopsy of a lymph node will substantiate the diagnosis and permit treatment at the earliest possible time. Carcinomas are rare in children, but when present may involve the bone marrow.

Neuroblastoma is the principal tumor in childhood, other than lymphoma, which produces bone marrow metastases. Tumor cells may be scattered throughout the marrow or may seem to replace the normal marrow elements, appearing singly or in groups suggestive of the "pseudorosettes" which are more characteristic of biopsy material, other than bone marrow. Malignant cells may not be found in every slide or even every bone marrow biopsy because of the patchy distribution of infiltration. A negative result does not necessarily exclude the possibility of marrow metastases. Bone marrow examination occasionally has been the means of establishing the diagnosis, as the following case summary illustrates.

CASE III. In July, 1952, a 3 year old girl was noted to be increasingly irritable and listless and to have increasing difficulty in seeing and walking. She suffered a convulsion, for which she was admitted to a local hospital. Roentgenograms of the skull showed a neoplastic destructive processs of the sphenoid and a soft tissue mass projecting into the posterior pharynx. Lumbar puncture was normal. She was referred to the Tumor Therapy Clinic in September, 1952, at which time she was blind and had a thick, mucoid, brownish nasal discharge. The remainder of the physical examination was negative. Blood studies revealed a hemoglobin of 11.2 gm., an erythrocyte count of 5.69 million per cubic milliliter, leukocytes 2200 per cubic milliliter, and a platelet count of 180,000 per cubic milliliter. A bone marrow biopsy revealed metastatic neuroblastoma (Fig. 31).

Lipoprotein Metabolic Disorders

In eosinophilic granuloma and Hand-Schüller-Christian disease, bone marrow involvement is localized, and eosinophils or reticulum cells may be prominent in these areas. In Letterer-Siwe disease the marrow usually appears normal, but occasionally increased numbers of large mononuclear cells are present, confirming the diagnosis. The diagnosis of Gaucher's disease may be made on finding characteristic marrow cells with bulky, faintly reticulated "tissue paper" cytoplasm and one or more relatively small nuclei (Fig. 32). Foam cells with abundant vacuolated cytoplasm (Fig. 33) confirm the clinical diagnosis of Niemann-Pick disease.

Biopsy of bone marrow is preferable to splenic puncture, which may be a hazardous procedure in children. The following history illustrates the value of bone marrow studies in the diagnosis of Gaucher's disease.

CASE IV. A son of Jewish parents was first seen in December, 1949, at the age of 7½ years. His past history was one of frequent upper respiratory infections. From the age of 5 years he had been observed to become pale and seemed increasingly more listless and tired. He was treated for anemia with vitamins and folic acid, with

no clinical improvement, but did respond briefly to parenteral iron and liver injections. When seen here, he showed no lymphadenopathy or bone symptoms. His liver was enlarged 6 cm. below the costal margin, and his spleen reached to his umbilicus. Repeated blood studies showed hemoglobin values between 12 and 13 gm., leukocyte

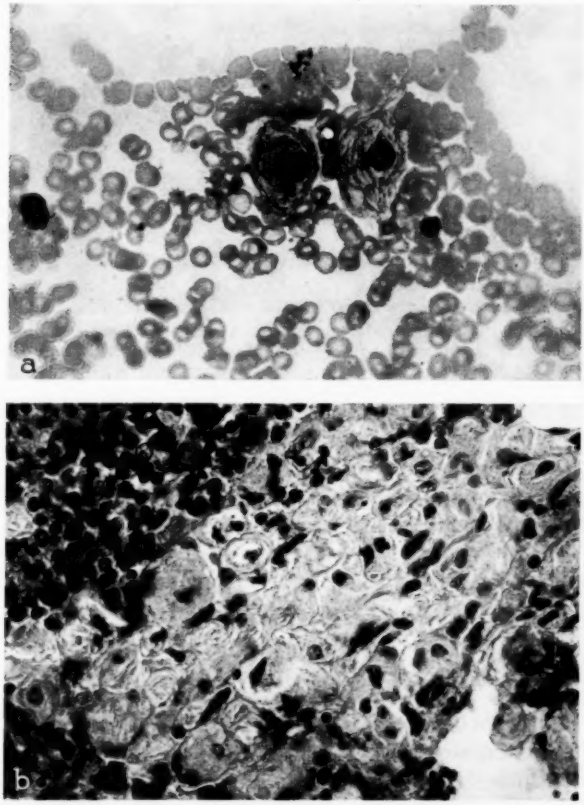

Fig. 32. Gaucher's disease. *a*, Marrow spread. The two adjacent Gaucher cells are large in comparison to the erythrocytes. × 800. *b*, Section of bone plug. An aggregate of Gaucher cells has tended to crowd the normal marrow. × 800.

count 5000 to 7000 per cubic milliliter and a platelet count of 67,000 to 87,000 per cubic milliliter. The differential count showed 42 to 49 per cent lymphocytes. A bone marrow examination revealed a large number of Gaucher's cells (Fig. 32).

INDICATIONS FOR SUPPORTIVE MARROW STUDIES

Leukemoid Reactions

A leukemoid blood picture presents an elevated leukocyte count with young forms and even occasional blast forms. It is often seen during the

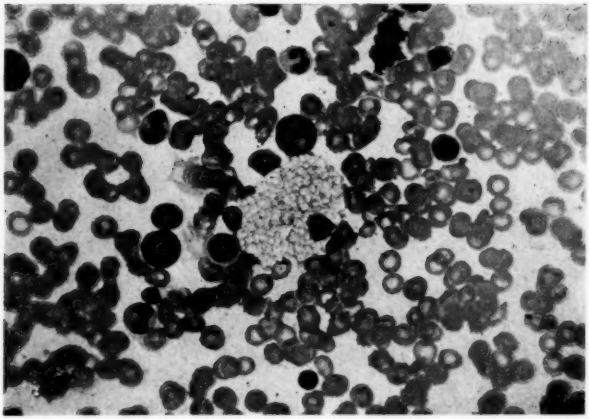

Fig. 33. Niemann-Pick disease. Marrow spread. A cell characteristic of this disease is surrounded by normal marrow cells. × 800.

course of infections such as pneumonia, meningitis, tuberculosis, pertussis, infectious mononucleosis or septicemia; with intoxication from burns, metallic poisoning, insect bites or carcinomas. When there is no obvious cause for an increased leukocyte count, particularly if immature forms are seen in the peripheral blood, leukemia must be suspected. In most instances the leukemoid reaction is easily distinguished from leukemia by the presence of well differentiated cells with no more than 20 per cent blasts in the bone marrow.

Nutritional Anemia Secondary to Iron Deficiency

The peripheral blood smear usually is classical. The anemia is hypochromic and microcytic. However, in the presence of hepatosplenomegaly and infection, it often is necessary to differentiate severe iron deficiency from early leukemia by bone marrow examination. The use of iron stains on marrow smears demonstrates the complete absence of iron in iron deficiency anemia.

Agranulocytosis

Agranulocytosis may occur secondary to infection or drug therapy, and is exemplified by a peripheral neutropenia and a bone marrow depleted of granulocytic precursors, but of otherwise normal cellularity. Bone marrow studies are useful in the differential diagnosis of agranulocytosis and leukemia, and may be valuable in following the granulocytic response to therapy.

Infectious Diseases

Infectious Mononucleosis. This condition frequently has been confused with leukemia, owing to the immature appearance of the nucleus of the atypical lymphocyte, especially when the disease is associated with thrombocytopenia. Supportive marrow studies to exclude malignancy may be essential when results of the usual peripheral blood examinations are not clear-cut. The marrow usually is hypocellular with a large number of atypical mononuclear cells of the lymphocytic and monocytic series. Heterophile antibody reactions are positive in only 30 per cent of cases with obvious signs and symptoms.[1]

Infectious Lymphocytosis. This disease is at times confused with lymphatic leukemia, owing to the presence of a leukocytosis with 90 to 100 per cent lymphocytes. The child's healthy appearance and the paucity of physical signs and symptoms are substantiated by a normal distribution of precursor cells in the bone marrow. The percentage of lymphocytes in the marrow differential may be increased, but the cells are of the small mature variety.

Hemoglobin and Erythrocyte Abnormalities

In these conditions the abnormalities in the marrow are in the red cell series. Congenital defects such as ovalocytosis and the sickle cell phenomenon may be detected in the marrow as well as in the peripheral blood.

Sickle Cell Anemia. Bone marrow shows marked erythrocytic hyperplasia. Marrow studies are rarely needed for diagnosis, but may be used to establish the presence or absence of other diseases.

Cooley's Anemia and Cooley's Trait. The general appearance of hypochromia and microcytes with anisocytosis and poikilocytosis in smears of the peripheral blood often is difficult to differentiate from simple iron deficiency anemia. Hemosiderin stains of the bone marrow show the absence of siderocytes in the iron deficiency, and large quantities of storage iron in Cooley's anemia or Cooley's trait.

Congenital Hemolytic Anemia. The diagnosis usually is made on the presence of spherocytes in the peripheral blood and the palpable spleen, in association with a family history of the disease. When a family history is not available, bone marrow examination often is helpful in excluding other disturbances in the red cell series.

Rarer Marrow Involvement

The marrow may show a predominance of immature granulocytes, but there is no consistent change of diagnostic value.

1. Protozoa, such as *Plasmodia* and *Leishmania,* occasionally may be found in the bone marrow.

2. The marked eosinophilia of both peripheral blood and marrow associated with parasitic infections such as ascariasis, filariasis, necatoriasis, ancylostomiasis and trichiniasis, particularly in this country, often is of aid in diagnosis.

3. In certain bacterial and fungal infections such as typhoid fever, tuberculosis, brucellosis, histoplasmosis and coccidioidomycosis, microorganisms may be seen in the marrow or, more often, isolated from cultures of marrow. Marrow cultures occasionally are of diagnostic value in those instances when peripheral blood cultures have been persistently negative.

Hypersplenism

Overactivity of the destructive power of the spleen may develop, owing to numerous variable causes such as hyperplasia and hypertrophy, obstructive splenomegaly, or diseases directly involving this organ. The peripheral blood shows evidence of depression of all elements, while the marrow shows production of normal cells in adequate quantity. This condition is seen in Banti's syndrome as well as in the secondary type of hypersplenism found in Cooley's anemia, chronic neutropenia and acquired hemolytic anemia.

Systemic Lupus Erythematosus

This disorder is occasionally encountered in pediatric practice and may present a problem in differential diagnosis. Formerly the L.E. phenomenon was observed chiefly in bone marrow preparations, but is now considered to be almost always positive in clotted peripheral blood.[4]

VALUE OF BONE MARROW STUDIES IN RELATION TO THERAPY

Megaloblastic Anemia

The response to vitamin B_{12} therapy occurs rapidly, and the bone marrow shows complete reversion to normal in a short time. Adequate initial treatment is achieved when the maximum reticulocyte response is reached, and the marrow appears completely normal. Changes in maintenance therapy are based on the appearance of abnormal young erythrocytic and giant band cells, which may be found in the marrow before any clinical signs appear or peripheral blood anemia occurs.

Leukemia

Bone marrow response indicates the degree of success of therapy in leukemia, and is a valuable aid in guiding the type and amount of

treatment. The chemical agents in use or under study may be potential bone marrow depressants, but with proper care and knowledge toxic effects may be avoided. Bone marrow aspiration and biopsy are invaluable guides in this respect, as illustrated by the following history.

CASE V. Diagnosis of acute leukemia had been established in an 11 year old girl in June, 1954. She was started on specific treatment with a purine antagonist in July, 1954. After an initial rise in the leukocyte count from 7000 to 10,000 to 20,000 per cubic milliliter, there was an abrupt fall to 2000 to 3000 per cubic milliliter in the second week, and below 1000 per cubic milliliter during the third week of treatment. Bone marrow spreads made at this time showed few cells. Aplasia of the marrow might have been suspected on the basis of this information alone. However, the plug and clot preparations demonstrated focal areas of marrow cells, many of which were blasts. Treatment, therefore, was continued.

SUMMARY

Bone marrow examinations are informative and diagnostic in many cases, but the marrow specimens must be properly prepared in order to be adequate. A combination of the more usual aspiration technique and biopsy gives satisfactory and, in many instances, essential information.

Marrow examinations are essential in the differential diagnosis of blood dyscrasias such as aplastic, hypoplastic and megaloblastic anemias, acute and chronic leukemia, Gaucher's disease and Niemann-Pick disease. They are also of primary importance in determining marrow infiltration from malignant lymphoma, neuroblastoma and other solid tumors.

Marrow examinations are not routinely indicated, but may be useful in the evaluation of leukemoid reactions, unusual cases of infectious mononucleosis, agranulocytosis, some nutritional anemias, infectious lymphocytosis, and to confirm the diagnosis of hypersplenism. Appropriate examination of biopsy material also may be useful in bacterial, fungal, protozoan and parasitic infections when the etiologic agent cannot be determined by the usual methods.

Marrow examinations are necessary to determine the response to therapy in megaloblastic anemia and leukemia. In the latter, the marrow is studied not only for evidence of remission, but also for signs of depressed hematopoiesis.

Aspirated marrow provides sufficient information in most instances when marrow examinations are indicated, but when aplasia is suspected, examination of a clot and bone plug is essential.

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REFERENCES

1. Bennike, T.: Penicillin Treatment of Infectious Mononucleosis. Arch. Int. Med., *87*:181, 1951.
2. Berk, N. G.: The Leukemoid Reaction. J. Albert Einstein Med. Center, 2:42, 1953.
3. Custer, R. P.: An Atlas of the Blood and Bone Marrow. Philadelphia, W. B. Saunders Company, 1949.
4. Hargraves, M. M.: Systemic Lupus Erythematosus and the L.E. Cell Phenomenon. Postgrad. Med., *16*:163, 1954.
5. Limarzi, L. R., Paul, J. T., and Bedinger, P. L.: Unusual and Diagnostic Bone Marrow Patterns, in Studies in Medicine. Springfield, Ill., Charles C Thomas, 1951, p. 262.
6. Smith, C. H.: Bone Marrow Examination in Blood Disorders of Infants and Children. M. Clin. North America, *31*:525, 1947.
7. Smith, C. H.: Hypoplastic and Aplastic Anemias of Infancy and Childhood: With a Consideration of the Syndrome of Non-hemolytic Anemia of the Newborn. J. Pediat., *43*:457, 1953.
8. Turkel, H., and Bethell, F. H.: Biopsy of Bone Marrow Performed by a New and Simple Instrument. J. Lab. & Clin. Med., 28:1246, 1943.
9. Zuelzer, W. W., and Ogden, F. N.: Megaloblastic Anemia in Infancy. Am. J. Dis. Child., *71*:211, 1946.

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BLOOD PRESSURE MEASUREMENTS IN INFANTS AND CHILDREN

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In children, as in adults, measurement of the blood pressure is an integral part of the physical examination. Though it is only one of the techniques for determining the adequacy of the circulation, there is no other simple way of obtaining the vital information which the sphygmomanometer yields. The techniques are more complex than those required in dealing with adult patients, owing to the variation in limb size, the change in normal values according to age, and the often less than complete cooperation of the patient; but with care, accurate and reproducible results may be obtained.

APPARATUS

Mercury manometers are the most reliable type of sphygmomanometers, although less compact than the aneroid type. In using a mercury manometer, frequent checks should be made to see that the surface level of the mercury is at zero and that the airways are well open throughout. An aneroid type of manometer should be checked against a mercury manometer at yearly intervals, at least. In both types the exhaust valve should function smoothly.

The size of the inflatable bag is of critical importance in pediatrics, and errors of 25 mm. of mercury or more can be produced by a cuff of improper size.[12, 14] For the newborn, a cuff 2.5 to 3 cm. in width should be used, and graduated sizes up to 18 cm. may be obtained commercially. At least the following sizes should be available to the examining physician: 3-, 5-, 8-, 12- and 18-cm. widths. When the

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proper size is not available, a larger cuff may be folded to a more appropriate width. As a general rule, the cuff should cover approximately two thirds of the length of the upper arm or leg. There is probably less chance for error if the bag completely encircles the limb. When the bag is properly applied, inflation should cause no bulging or displacement.

TECHNIQUE

If possible, a brief explanation of the procedure beforehand may increase the cooperation of children. Often an older child may be distracted by watching the up and down movements of the mercury column when pointed out to him. Infants sometimes can be pacified by a bottle of milk. In other infants the blood pressure can be determined accurately only under sedation (barbiturates, chloral hydrate or morphine).

Arm pressures may be determined with the patient sitting or lying. Leg pressures are most conveniently obtained with the patient prone.

The bag should be completely deflated and applied evenly and snugly around the upper arm or leg with the lower edge about 1 inch from the antecubital or popliteal fossa. If there is evidence of venous engorgement, the limb should be elevated before the cuff is inflated, to promote drainage.

Palpation

The first practical method of determining blood pressure, the palpatation method, was first described by Riva-Rocci in 1896.[11] In using a standard sphygmomanometer set, the cuff should be inflated in steps of 10 mm. of mercury until the radial or brachial pulse can no longer be palpated. Then by releasing the pressure in the manometer by 2 to 3 mm. of mercury per heart beat, the point of reappearance of the pulse is observed. This point usually is 5 to 10 mm. lower than the systolic pressure determined by auscultation. This method routinely should be used as a preliminary to the auscultatory determination, because it (*a*) gives an approximation of the pressure needed in the system for subsequent determination, (*b*) offers reassurance to the patient, and (*c*) avoids errors due to auscultatory gap.* Indeed, in infants when auscultation is extremely difficult, the palpation method is frequently the only practical method.

With considerable experience an approximation of the systolic blood pressure may be made, in the absence of a readily available sphygmo-

* Auscultatory gap is an auscultatory phenomenon caused by venous congestion, in which the sounds disappear for an interval between systolic and diastolic levels.

manometer, from the amount of pressure needed to obliterate the radial pulse by a manual compression of the brachial artery. Needless to say, this impression should always be checked by an accurate determination with a sphygmomanometer as soon as possible.

Auscultation

Korotkow,[9] in 1905, first described the four phases of sounds, produced by gradually lowering the pressure in a sphygmomanometer, audible with a stethoscope at the antecubital fossa. Only two phases, however, are of clinical importance.

The sphygmomanometer cuff should be rapidly[10] inflated to a level approximately 30 mm. of mercury over the systolic level obtained by palpation, and the pressure released at a rate of 2 to 3 mm. of mercury per beat. The level at which the first sound (first phase of Korotkow) is heard should be recorded as the systolic pressure. (This level is actually an average of 3 to 4 mm. of mercury lower than the pressure obtained by direct measurement with an intra-arterial needle.) As the level continues to fall, just prior to the complete disappearance of sounds, the sounds become muffled. This level was the fourth phase described by Korotkow, and on the basis of oscillometric studies this was thought to correspond to the diastolic blood pressure. However, on direct measurement, Hamilton, Woodbury and Harper[8] found that this level was 9 mm. of mercury too high on the average. Subsequently, in 1951, the American Heart Association[2] recommended that the point of complete cessation be taken as the best index of diastolic pressure.[13] Under certain conditions, however, in which no cessation of sounds occurs, the point of muffling should be taken as the diastolic pressure, and so recorded.

The rate of fall of the column of mercury is important. It should not be so fast as to cause a negative pressure above the mercury column, nor so slow as to cause trapping of blood between the systolic and diastolic levels.

Variations in the pressure and position of the stethoscope diaphragm or bell can make considerable difference in the distinctness and quality of sounds. Excessive pressure must be guarded against especially in determining diastolic pressures, because of the easy compressibility of youthful arteries causing erroneously low readings.

In measuring the blood pressure in the legs, the correct size of cuff is absolutely essential. Although systolic pressures in the thigh are normally 10 to 40 mm. of mercury higher than in the arm, the diastolic pressures are essentially the same.[8] This may be used as a guide when in doubt as to whether the cuff used for the thigh was of proper width. Determination of pressure in the legs is mandatory in any child with

evidence of congenital heart disease, hypertension or diminished femoral pulses.

If special studies of blood pressure are required, the readings should be taken under basal conditions, before breakfast and preferably before arising in the morning.

Oscillometry

Oscillometry was first used by Erlanger in 1904.[4] The principle is based on a system with low inertia, transmitting and magnifying the pulsations which occur when the pressure inside the arterial wall exceeds the cuff pressure. The points of onset and disappearance of these pulsations are regarded as approximations of the systolic and diastolic pressures. Our experience at Children's Hospital with this method has been disappointing, and it appears to offer little advantage in the pediatric field. However, the onset of oscillations in an ordinary mercury or aneroid manometer frequently appears 4 to 5 mm. above the level of the first sounds and therefore offers an advance warning.

Flush Method

Although the application of this technique to pediatrics was reported as late as 1952 by Goldring and Wohltmann,[6] it is based on a principle outlined in 1899 by Gaertner,[5] who utilized a miniature cuff to determine digital blood pressure. Although it is only an approximation of true systolic pressures, in struggling newborn infants it is frequently the only method of use. With care, the results are reproducible and reasonably reliable. Also, in coarctation of the aorta when the sounds in the legs are inaudible and no pulses palpable, a comparative pressure may be obtained with this method.

An appropriate-sized cuff is placed around the ankle or wrist. Elevation of the limbs, and mild local pressure on the palms or soles sufficient to cause blanching, are maintained while the cuff pressure is being elevated. With the limbs at heart level the pressure in the cuff is released, at a rate of 2 to 3 mm. of mercury per beat. The point at which a flush appears in the blanched areas is recorded as the blood pressure, but with the added notation, "by flush method." In a series of twenty-five children of varying ages the flush pressures were considerably below systolic pressures, with an average of 25 mm. of mercury difference. This level closely approximates the mean pressure of these children, since the average pulse pressure of the group is 46 mm. of mercury. In one patient direct measurement of arterial mean pressure (using an electronic integrator) agreed within 2 mm. of mercury with the flush pressure taken simultaneously on the other arm. In pa-

tients with coarctation, in whom no sounds can be heard in the legs, the flush pressure in the arms is moderately but definitely higher than in the legs.

NORMAL VALUES

The most reliable work on normal values was done by Graham,[7] who made 25,000 determinations on 3580 school children in a long-term study. Using the point of complete cessation as diastolic pressure, and cuff widths that covered no more than two thirds of the upper arm, his results seem more valid than those of any comparable series. His series covers the ages five through sixteen years of age. There is a gradual

TABLE 14. *Normal Blood Pressure Values*

AGES	MEAN SYSTOLIC ± 2 S.D.*	MEAN DIASTOLIC ± 2 S.D.*
5	94 ± 14	55 ± 9
6	100 ± 15	56 ± 8
7	102 ± 15	56 ± 8
8	105 ± 16	57 ± 9
9	107 ± 16	57 ± 9
10	109 ± 16	58 ± 10
11	111 ± 17	59 ± 10
12	113 ± 18	59 ± 10
13	115 ± 19	60 ± 10
14	118 ± 19	61 ± 10
15	121 ± 19	61 ± 10
16	121 ± 19	61 ± 10

* Mean value plus or minus 2 standard deviations includes 95% of all normal subjects.

increase in systolic blood pressure, and an apparent increase in variability with increasing age. These changes are exaggerated at puberty, and continue during adolescence. In general, there was agreement of average levels between male and female, within 1 mm. of mercury.

Another series by Downing[3] on normal girls from three to sixteen years of age is less acceptable, because only two sizes of cuffs were used, 9.5 and 13 cm., and the point of muffling was taken for the diastolic pressure.

In the age range below five years there are no series as statistically reliable as Graham's. Woodbury, Robinow and Hamilton[14] measured directly the blood pressure in twenty-four newborn infants and found an average systolic pressure of 80 mm. of mercury, with a standard deviation of 8.2 mm. The systolic pressures rose 10 to 15 mm. of mercury gradually over the first ten days of life. They also found that pressures in premature infants were considerably lower, in apparent direct proportion to their fetal age, a five month fetus having a pres-

sure of 39/21 mm. of mercury; a six and one-half month fetus 55/25 mm.; a seven month fetus 70/35 mm.; and an eight month fetus 75/45 mm. of mercury. These investigators also found that crying in infants raised the systolic pressure from 10 to 45 mm. of mercury, with an average increase of 27 mm.

In a series studied by an English investigator, Allen-Williams,[11] with appropriate-sized cuffs, but using the point of muffling as the diastolic pressure, and involving only a modest number of children, the systolic pressure rose gradually from 91 mm. of mercury at less than one year, to 99 mm. at age four to five. He also found a high degree of correlation between systolic and diastolic pressures, and could usually predict diastolic from the systolic pressure.

INTERPRETATIONS

Interpretation of blood pressure measurements is limited at once by errors of technique, by relatively wide fluctuations possible in normal infants, and by the range of normal values varying with age. When an apparently abnormal blood pressure is found, after checking for errors of technique, the patient should be allowed to rest and to adjust to the examiner and the environment, and, if possible, the pressure should be checked under basal conditions. The results, finally, must be interpreted with reference to the normal range for a given age. For example, the normal range of systolic pressure for a six year old child may be reasonably defined as the average systolic for that age, 100 mm. of mercury, plus or minus twice the standard deviation: 7.5 mm. of mercury, or 100± 15 mm. of mercury (see Table 14). This would include 95 per cent of all normal subjects. If, in a child that age, the blood pressure falls outside the range of 85 to 115 mm. of mercury by a significant margin, it probably indicates disease involving the cardiovascular system.

If the blood pressure remains significantly elevated, a cause must be sought. In a rough order of frequency, in pediatrics, the causes of hypertension may be (1) acute or chronic nephritis; (2) coarctation of the aorta; (3) widened pulse pressure, as in aortic regurgitation, patent ductus arteriosus and thyrotoxicosis; (4) central nervous system diseases, including poliomyelitis, encephalitis and tumors; (5) poisoning with mercury or lead; (6) pituitary and adrenal tumors, both cortical and medullary (pheochromocytomas); and (7) essential hypertension.

Low blood pressure is most commonly found, in pediatrics, in shock, from (*a*) loss of blood, plasma or extracellular fluids (diarrhea and vomiting); (*b*) inadequate cardiac output, as in Adams-Stokes syncope, cardiac tamponade or acute cardiac failure. Another cause of

acute hypotension is vasovagal syncope, or, simply, fainting. Chronic hypotension is occasionally seen in adrenal insufficiency, hypothyroidism and constrictive pericarditis.

Additional information can be obtained with the sphygmomanometer. Pulsus paradoxus, too slight to be determined by palpation alone, can be picked up with careful auscultation, as the systolic pressure rises slightly in expiration, as opposed to the normal rise in late inspiration. Pulsus alternans may also be detected by maintaining the cuff pressure at systolic level for a brief interval.

Finally, the pulse pressure may be calculated simply by subtracting the diastolic from the systolic pressure. Wide pulse pressure, which may be detected usually by the bounding or water-hammer character of the radial pulse, is seen (1) after exercise, and in thyrotoxicosis, anemia, fever and vasodilatation; (2) essential hypertension; (3) aortic regurgitation, patent ductus arteriosus and arteriovenous fistulas; and (4) heart block with bradycardia. Diminished pulse pressure is found in (1) shock, (2) severe mitral and aortic stenosis, (3) pericardial effusion and constrictive pericarditis, and (4) heart failure.

REFERENCES

1. Allen-Williams, G. M.: Pulse-Rate and Blood Pressure in Infancy and Early Childhood. Arch. Dis. Childhood, *20*:125, 1945.
2. American Heart Association: Recommendations for Human Blood Pressure Determinations by Sphygmomanometers. New York, 1951.
3. Downing, M. E.: Blood Pressure of Normal Girls from Age Three to Sixteen Years of Age. Am. J. Dis. Child., *73*:293, 1947.
4. Erlanger, J.: A New Instrument for Determining the Minimum and Maximum Blood Pressures in Man. Johns Hopkins Hosp. Rep., *12*:53, 1904.
5. Gaertner, G.: Ueber einen neuen Blutdruckmesser. Wien. klin. Wchnschr., *12*: 696, 1899.
6. Goldring, D., and Wohltmann, H.: Flush Method for Blood Pressure Determinations in Newborn Infants. J. Pediat., *40*:285, 1952.
7. Graham, A. W., Hines, E. A., Jr., and Gage, R. P.: Blood Pressure in Children between the Ages of Five and Sixteen Years. Am. J. Dis. Child., *69*:203, 1945.
8. Hamilton, W. F., Woodbury, R. A., and Harper, H. T.: Physiologic Relations between Intrathoracic, Intraspinal, and Arterial Pressure. J.A.M.A., *107*:853, 1936.
9. Korotkow, N. S.: Mitt. Klin. Med. Akad. (St. Petersburg), *11*:365, 1905. See also Arch. Int. Med., 1910.
10. Ragan, C., and Bordley, J.: Measurements of Blood Pressure. Bull. Johns Hopkins Hosp., *69*:526, 1941.
11. Riva-Rocci, S.: Gazz. med. di Torino, *51*:52, 1896.
12. Robinow, M., Hamilton, W. F., Woodbury, R. A., and Volpitto, P. P.: Accuracy of Clinical Determinations of Blood Pressure in Children. Am. J. Dis. Child., *58*: 102, 1939.
13. Steele, J. M.: Measurements of Arterial Pressure in Man. J. Mt. Sinai Hosp., *8*: 1049, 1941–42.
14. Woodbury, R. A., Robinow, M., and Hamilton, W. F.: Blood Pressure Studies on Infants. Am. J. Physiol., *122*:472, 1938.

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BLOOD PROTEIN MEASUREMENTS IN DIAGNOSIS OF DISEASE

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The purpose of this paper is to present a brief review of blood proteins: their known functions, normal concentrations, and changes in various diseases. Knowledge of their concentrations is helpful in pediatric practice in the diagnosis of disease, in estimating prognosis and severity and in understanding physiologic changes.

BLOOD PROTEIN ORIGINS AND FUNCTIONS

Since serum albumin and serum globulin are distinct from chemical, physical, physiologic, immunologic and clinical standpoints, they must for the most part be considered individually. The bulk of evidence to date favors the liver as the primary site of formation of serum albumin. Less is known of the origin of globulin, although the evidence suggests that its origin may be in the plasma cells and the reticuloendothelial system everywhere. Physically, the globulin molecule is about two and one-half times as large as that of albumin. The osmotic influence of serum albumin is about four times that of the globulin. Physiologically, albumin is of much greater importance than globulin in the maintenance of blood volume and fluid balance. The concentration of albumin in the blood fluctuates more than does that of globulin. In contrast to globulin, albumin is more easily affected by dietary intake of protein. The globulin concentration rises much more quickly after hemorrhage than does albumin.

The proteins, especially globulin, contribute a certain viscosity to the

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blood, a factor in maintenance of blood pressure. Also, by means of their buffering capacity, the proteins aid in regulation of acid-base balance. Certain vitamins, minerals and lipids depend upon the proteins for transport. The role of fibrinogen in the clotting of blood and the association of gamma globulin with immune reactions are well known.

BLOOD PROTEIN CONCENTRATIONS

Just as a patient usually has only one disease at a time, usually only one fraction or the other—that is, either total albumin or total globulin—is involved. Furthermore, in disease, the total globulin concentration is rarely decreased below normal and the albumin concentration is almost never increased above normal.[19] Therefore a rise in total protein concentration generally indicates a rise in total globulin, and a decrease in total protein indicates a decrease in albumin concentration. The principal exception* to this is in the premature infant, in whom both total globulin and albumin are characteristically lower than in other infants.

The term "albumin-globulin ratio" has held a place in the vernacular of medicine since Epstein first called attention to the clinical significance of blood proteins in 1912.[8] The use of the albumin-globulin ratio is misleading, however, and should be discarded. The ratio gives no conception of the actual change in either the albumin or the globulin concentration. For example, the albumin-globulin ratio may be reversed in lymphopathia venereum; it is likewise reversed in nephritis. In lymphopathia venereum, however, this is due to an increase in globulin; in nephritis it is due to a decrease in albumin. What we need to know, therefore, is the absolute concentration of albumin and globulin, not the albumin-globulin ratio.

The total serum protein concentration† in premature infants[7, 15, 24, 31, 33-35, 40, 41] ranges from 5 to 6 gm. per 100 ml. at birth. However, it decreases during the first month to an average low of about 4.5 gm. (range 4.1 to 4.7). Infants of lower weight seem to have lower levels than those of higher weight. The protein level may not begin to rise until after sixty to seventy days of age.[34, 40]

The total serum protein in full term infants ranges from 5.1 to 6.4 gm. per 100 ml. at birth. The protein level then falls slowly for about six weeks to an average low of about 5.4 gm. (range 4.7 to 7.4) and then slowly begins to increase.[15, 34, 40]

The serum albumin concentration in premature infants ranges

* Also in early severe hemorrhage, far advanced cirrhosis and the rare cases of agammaglobulinemia and hypogammaglobulinemia.

† Values vary somewhat in different studies and with different methods.

between 3.2 and 4.6 gm. per 100 ml. (average about 4 gm.). It then falls to an average of about 3 gm. at thirty days of age.[33]

In full term infants, plasma* albumin averages 4.8 gm (±0.47 S. D.) during the first few days and is statistically higher than in the premature infant.[31] At three to twelve weeks it averages about 3.5 gm.[31, 40]

In premature infants, during the first few days, total globulin averages 1.6 gm. per 100 ml. (±0.37 S. D.).[31] It then falls so that during the first twelve weeks total globulin ranges from 1.0 to 1.6 gm. per 100 ml. (mean 1.4). There is a slight tendency for prematures of lower body weight to have lower globulin levels than those of higher body weight. The levels are significantly lower than in full term infants.[2, 24, 35, 40, 41]

Total globulin averages 1.75 gm. per 100 ml. (±0.49 S. D.) in full term newborns during the first few days.[31] From birth to two weeks the globulin averages 2.41 gm. (±0.69 S. D.). The average level then falls slightly to a low of 1.68 (±0.55 S. D.) between four and six weeks of age and rises thereafter to an average of 2 gm. per 100 ml. (±0.38 S. D.) between three months and one year.[31, 34, 40]

In premature infants, alpha globulin levels vary from 0.2 to 0.5 gm. per 100 ml.[33] Infants heavier than 1400 gm. tend to have slightly higher values than lighter infants. No change has been noted with increasing age up to two months.

The concentration of beta globulin in premature infants ranges from 0.5 to 0.8 gm. per 100 ml.[33] No correlation was found with age or weight of the infants.

There is a distinct decrease in average gamma globulin of premature infants, with increasing age: 0.7 gm. per 100 ml. during the first week to about 0.2 gm. at forty days. There is no tendency to rise by sixty days of age.[33] One investigator reported that it continues to decrease gradually until three to five months, after which a slow increase occurs.[15] It may not normally reach adult levels until five years of age.

In general, the adult values of blood proteins are reached at about one year of age. Normal values over one year of age are as follows: total protein, 6.0 to 7.5 gm. (average 6.7); albumin, from 3.0 to 4.5 gm. average 4.0); and total globulin, from 1.5 to 3.0 gm. per 100 ml. (average 2.5). Alpha globulin varies normally from 0.7 to 1.3 gm. per 100 ml.; beta globulin from 0.6 to 1.3; gamma globulin 0.7 to 1.3; and fibrinogen 130 to 250 mg. per 100 ml.

* Some investigators report plasma values and others serum values. Although plasma protein contains about 0.2 gm. per 100 ml. of fibrinogen, whereas serum does not, the total protein concentrations of the two are about the same. The reason for this appears to be that the anticoagulant added to make plasma (i.e., citrate or oxalate) withdraws water from the red cells, and the result is dilution of the protein to an extent which roughly corresponds to 0.2 gm. per 100 ml.[18] Therefore, for practical purposes, plasma and serum values may be used interchangeably except for fibrinogen. The latter, of course, can be determined only in plasma.

BLOOD PROTEIN CHANGES IN DISEASE

Little information is available about the changes in these protein fractions in newborn and premature infants with various illnesses.

Hypoproteinemia

With the few exceptions noted, hypoalbuminemia and hypoproteinemia are synonymous. Pediatric conditions in which hypoalbuminemia is found are listed in Table 15.

TABLE 15. *Hypoproteinemia*[23] (*Hypoalbuminemia*)

1. MALNUTRITION
   - Dietary
     - (a) Endemic and sporadic
     - (b) Associated with chronic infection, pellagra, beriberi, etc.
   - Poor absorption
     - (a) Idiopathic?
     - (b) Diarrhea, celiac disease, sprue
     - (c) Intestinal fistulas
     - (d) Cancer of stomach, pancreas
   - Poor utilization?
     - (a) Pernicious anemia, uncontrolled
     - (b) Diabetes mellitus, unregulated
2. KIDNEY DISEASES
   - Nephrosis—all types
   - Glomerular nephritis, chronic
   - Amyloid kidney
3. LIVER DISEASES
   - Cirrhosis
   - Cancer
   - Hemochromatosis
4. PROTEIN DILUTION
   - Excess fluid administration
5. PROTEIN LOSS
   - Hemorrhage, acute or chronic
   - Weeping wounds, burns or other skin lesions
   - Shock, surgical and traumatic
6. HEART FAILURE
7. HYPERTHYROIDISM
8. CHRONIC POISONING
   - Benzene, carbon tetrachloride
   - War gas (phosgene)
   - Phosphorus

Malnutrition. One of the most common causes of hypoproteinemia is malnutrition. This may be due to a diet poor in protein or to improper absorption or utilization of a diet adequate in protein content. Poor absorption may occur in diarrhea, celiac syndrome, cancer of the

stomach or pancreas, intestinal fistulas, and so on. In diarrhea, serum protein may, in addition, be lost from the body in the stools. Even if properly absorbed, serum protein may not be synthesized if the patient has any form of liver disease.

Abnormal metabolism of protein may be found in uncontrolled diabetes mellitus, pernicious anemia or hyperthyroidism. When diabetes is uncontrolled, the serum protein concentration is often low; when controlled, it usually returns to normal. A diabetic whose serum proteins are low without other cause is probably not completely regulated. In hyperthyroidism the serum protein concentration may be low, but it generally returns to normal after adequate chemotherapy or surgery. In hyperthyroidism increased blood volume as well as increased catabolism may be responsible for the low serum protein concentration.[20]

Cancer,[20] whether primary or metastatic, is usually accompanied by low serum albumin concentration when it affects the stomach, pancreas or liver. When the cancer does not affect these organs, however, serum albumin is usually normal in spite of marked tissue emaciation. Perhaps cancer of the stomach and pancreas interferes in some way with the digestion of protein within the intestine. When it affects the liver, perhaps it interferes with anabolism of serum proteins from the absorbed amino acids.

Kidney Diseases. The lowest serum albumin concentrations are usually seen in the nephrotic syndrome. Contrary to the impression given by frequent reference to the reversed albumin-globulin ratio, the total globulin is often not above normal levels in this disease.

Heart Failure. In the presence of uncomplicated heart failure[22] there is usually some lowering of the total protein concentration, but it is rarely below 5.5 gm. per 100 ml. This slight decrease may be due to the increased blood volume which accompanies heart failure, or to decreased liver function as a result of chronic passive congestion. Not infrequently, when a child has peripheral edema (and more commonly in the adult), there is a question whether it is due to heart failure with passive congestion of the kidneys or to chronic glomerulonephritis. If the total protein is above 5.5 gm., or the albumin above 3 gm., the probability is that heart failure caused the edema. If the proteins are below these levels, the edema is probably due, at least in part, to the lowered serum albumin concentration such as is found in chronic glomerulonephritis.

Protein Loss. Serum protein may be lost from the body by hemorrhage, either acutely as in Meckel's diverticulum, or chronically as in ulcerative colitis. There may be loss of plasma alone as in weeping forms of eczema or in burns. Recently the importance of correction of hypoproteinemia, particularly in burns, has been stressed.

Chronic Poisoning. Certain causes of chronic poisoning, such as ben-

zene, carbon tetrachloride and phosphorus, produce hypoproteinemia, probably through their effect upon the liver.

Hyperproteinemia

Hyperproteinemia (above 7.5 gm.) (Table 16) almost invariably means hyperglobulinemia. In the author's experience, in which the

TABLE 16. *Hyperproteinemia in Pediatrics*[23] (*Hyperglobulinemia*)

1. DEHYDRATION
   - Insufficient intake
   - Fluid loss
   - (a) Intestinal obstruction and fistulas
   - (b) Diarrhea
   - (c) Vomiting
   - (d) Diabetic acidosis
   - (e) Burns
   - (f) Heat cramps and heat exhaustion
   - (g) Fulminant infections
   - (h) Addison's disease
2. DISEASES INVOLVING RETICULOENDOTHELIAL SYSTEM
   - Multiple myeloma
   - Monocytic leukemia
3. INFECTIONS, CHRONIC
   - Suppurative, various
   - Tuberculosis
   - Syphilis
   - Lymphopathia venereum
   - Subacute bacterial endocarditis
   - Leprosy
   - Boeck's sarcoid
4. PARASITIC INFECTIONS
   - Malaria
   - Visceral leishmaniasis
   - Schistosomiasis
   - Filariasis
   - Trypanosomiasis
5. FIBRINOID (COLLAGEN) DISEASES
   - Periarteritis nodosa
   - Lupus erythematosus
   - Rheumatoid arthritis
6. LIVER DISEASES
   - Cirrhosis of liver
   - Cancer, primary or metastatic

cases were selected because they were suspected of possible blood protein changes, hyperproteinemia was found in less than 10 per cent of all the analyses.[21]

Diseases Involving the Reticuloendothelial System. The reticuloendothelial system may be the site of origin of globulin, and diseases involving this system form a convenient group, each causing hyperproteinemia. Why a disease involving the probable site of origin of globulin should

cause an increase in its circulating concentration is not understood, although some plausible theories lend themselves to provocative thought and experiment. Of these diseases, monocytic leukemia, multiple myeloma and kala-azar (visceral leishmaniasis) cause the highest values for total serum protein concentration which have been observed. The total protein concentration in multiple myeloma may reach as high as 23.4 gm. per 100 ml. and in monocytic leukemia as high as 10.1 gm.[21]

Comparatively small increases in the serum globulin may be found in lymphoid and myeloid leukemia and in carcinomatous metastasis to bone marrow as well as to liver.

Chronic Infections. In most children hyperproteinemia is due to chronic infection, and this may be of a suppurative or nonsuppurative nature. These include bronchiectasis, lung abscess, tuberculosis, syphilis, chronic osteomyelitis, chronic pyonephrosis, Boeck's sarcoid, leprosy, lymphopathia venereum and subacute bacterial endocarditis. Except for lymphopathia venereum, subacute bacterial endocarditis, Boeck's sarcoid and pulmonary suppuration, the increase of globulin in these cases is not usually marked and the total protein concentration is seldom over 9 gm. per 100 ml. A few diseases which are rare in this country, such as filariasis, trypanosomiasis and schistosomiasis, may also produce increased globulin concentration.

It is doubtful whether the increase in globulin in these conditions is due entirely to increase in immune bodies in the blood. The increase may be the result of antibody-antigen interaction and may not be active antibody.[23]

Fibrinoid (Collagen) Diseases. It is interesting that the so-called collagen or fibrinoid diseases—periarteritis nodosa, lupus erythematosus and rheumatoid arthritis—are commonly associated with increased serum globulin concentration.

Summary. Thus we find hyperglobulinemia or hyperproteinemia in six different groups: (1) dehydration, (2) diseases involving the reticuloendothelial system, (3) chronic infections, (4) parasitic infections, (5) "fibrinoid" or "collagen" diseases, and (6) liver disease (Table 16). The total protein concentration is rarely above 9 gm. per 100 ml. in conditions other than multiple myeloma, lymphopathia venereum and dehydration and in a few less common diseases. The fact that it is increased above normal may be helpful in differential diagnosis[32] and in the treatment of dehydration.

Hypoalbuminemia and Hyperglobulinemia

A few diseases, or combinations of diseases, cause simultaneous hypoalbuminemia and hyperglobulinemia. *Acute glomerulonephritis* and *liver disease* (particularly cirrhosis) are the most common single causes of simultaneous reduction in the albumin and increase in the total

globulin concentration. Increased total globulin concentration is common in liver disease for reasons which are not clear, and the decrease in albumin may be due to disease of the liver, which is its probable site of origin. The decrease in albumin in acute glomerulonephritis is small. It is probably only partly due to loss in the urine and possibly also due to the effect of this generalized vascular disease upon the liver. The increase in globulin concentration in acute glomerulonephritis is probably secondary to the preceding streptococcal infection. The only other circumstance in which we have found such a change in both albumin and total globulin is that in which two conditions are present, the one causing hyperglobulinemia, the other hypoalbuminemia (for example, tuberculosis causing increased globulin, and malnutrition or nephritis causing decreased albumin).

BLOOD FIBRINOGEN

Slight increases in globulin above the usual for the individual but not above the upper limit of normal for the population occur frequently, particularly in infections. There may also be an increase in fibrinogen in these states. The increase of these proteins is believed to be responsible for the increased tendency of red cells to form rouleaux and thereby increase the erythrocyte sedimentation rate.

Congenital absence of fibrinogen, or *afibrinogenemia*, is a rare condition. There are only about twenty reported cases.[9] It is hereditary (probably recessive) and is not sex-linked. The defect appears to be a failure to synthesize adequate quantities of fibrinogen.[11] In this disease, clotting time is indefinitely prolonged. It is possible that the condition is more common than is supposed, since there is a tendency to classify male "bleeders" as hemophiliacs. The diagnosis may be missed if only bleeding times are performed (these are normal) or if clotting or prothrombin times are not carried to the end point. Also, perhaps some infants with afibrinogenemia die from umbilical or other hemorrhage without diagnosis.

Treatment of afibrinogenemia consists of transfusions with whole blood or plasma to keep fibrinogen above the critical point of 50 mg. per 100 ml. This may mean replacing one fourth of the patient's blood volume with normal plasma at weekly intervals. Frick and McQuarrie[9] found that a fibrinogen level of 161 mg. per 100 ml. after infusion of about 4 gm. of fibrinogen fell to zero in twelve days.

BLOOD PROTEIN FRACTIONS

Ultracentrifugation, electrophoretic, chromatographic, immunochemical and chemical methods have made possible studies of the concen-

tration of the various protein fractions. About thirty-five proteins have been accounted for, representing about 90 per cent of the total plasma protein. Possibly the remaining 10 per cent contains many more fractions. Albumin constitutes about two thirds of all the serum protein. Over half of the albumin is mercaptalbumin. The once familiar alpha, beta and gamma fractions of globulin have been separated into subfractions. Some of these are important in transport. Blood lipids are carried by alpha and beta lipoproteins, metals such as iron by a specific protein among the beta globulins, and copper and zinc by beta globulin. Protein-bound iodine and bilirubin, alkaline phosphatase and certain anterior pituitary hormones are wholly or in part associated with the alpha globulins. Various drugs such as sulfonamides and dyes such as Evans blue are transported in similar manner. Many of the hormones are proteins or protein combinations (e.g., insulin, thyroglobulin, parathyroid and growth hormones, adrenocorticotropin, and so on). Mucoprotein, C-reactive protein and other so-called phase reactants are the subjects of much current study. Fibrinogen appears to be a single component. However, prothrombin and a number of factors and cofactors, accelerators and inhibitors are circulating proteins which participate vitally in the clotting mechanism.

Gamma Globulin

Absence of gamma globulin (agammaglobulinemia) or its presence in amounts less than normal (hypogammaglobulinemia) appears to be associated with two different clinical syndromes. The former is characterized by lack of immunity and consequent susceptibility to repeated infections, and the latter by edema and even anasarca.

Agammaglobulinemia. The first case of agammaglobulinemia was reported by Bruton.[4] The boy, between the ages of four and a half and eight years, had eighteen bouts of sepsis with eight different types of pneumococci, five episodes of otitis media and three attacks of epidemic parotitis. Prophylactic antibacterial therapy was not always successful in preventing reinfection. After autogenous pneumococcal vaccine, typhoid vaccine and diphtheria immunization, no circulating antibodies could be detected. Moreover, no complement-fixing antibodies were present in the serum twenty-two days after the third episode of epidemic parotitis. The recurrent infections were controlled by repeated injections of gamma globulin.[5]

About nineteen patients with this syndrome have since been described,[6, 12, 17, 25, 29] all having recurrent infections that were most frequently caused by pneumococci, meningococci, staphylococci or *Hemophilus influenzae*.[5, 17] Fatal, generalized vaccinia has been reported[25] in an infant with complete absence of gamma globulin. Except for one

adult,[36] the entity has not been seen in a female. It is thought that agammaglobulinemia may be due to congenital hypoplasia of the lymphoid tissues.[6]

Hypogammaglobulinemia. This appears to be a congenital failure in the formation of gamma globulin and is associated with moderate lowering of the albumin concentration as well. Perhaps it is due to some defect in the liver, and it may be familial. These patients are generally remarkably free from infection, although the first reported patient succumbed to a combined ear and lung infection after surviving pertussis and an episode of pneumonia.[38] The patients have low to low-normal total globulin concentrations (about 1.1 to 1.6 gm. per 100 ml.), but have specifically low but apparently not absent gamma globulin concentrations. The total albumin concentrations are generally between 2.3 and 3.3 gm. per 100 ml. These patients characteristically have edema and even anasarca resembling the clinical picture of the nephrotic syndrome, yet have no albumin in their urine. Blood cholesterol is normal or only slightly elevated. Although the patients are not subject to repeated infections as in agammaglobulinemia, attempts to stimulate antibody formation by injection of diphtheria toxoid, typhoid vaccine and pertussis vaccine have failed to produce a positive Widal, pertussis antibodies or rise in serum proteins.[37] The Schick and Dick tests were, however, negative in one case.[37]

The few patients examined at autopsy have shown some abnormality of the liver, interpreted as atrophy of the hepatic cords or hepatitis. Some have had achlorhydria. In 1945 Schick and Greenbaum[37] presented the case of an eleven year old girl who had had episodes of edema since three months of age. Serum albumin was 3.3 gm. per 100 ml., total globulin 0.8, and there was "practically no migration" of gamma globulin in electrophoretic studies. Her mother gave a history of edema as a young adult, and she had a total globulin concentration of 1.1 gm. per 100 ml.

In addition to the reports of twelve apparently similar cases which were reviewed by Schick and Greenbaum, a few more cases have been reported. In contrast to agammaglobulinemia, both sexes are about equally represented. Perhaps many more have been seen. Kagan has observed three such cases. One of these patients had severe jaundice and died at another hospital. By personal communication it was learned that the patient had advanced cirrhotic changes at autopsy. There is a tendency in some of the patients to recover spontaneously.[10, 37, 39]

These cases are of added interest because in the nephrotic syndrome the gamma globulin is also characteristically low, although not quantitatively as much. In view of the similarity in edema in the two conditions, one wonders about the possible role of low gamma globulin in the nephrotic syndrome in the production of the edema in spite of the

long-accepted relationship between edema and the low albumin concentration in this syndrome.

Gamma globulin concentration is also low in Cushing's syndrome. Here it is presumed that the excess of adrenocortical hormones has exhausted the lymphoid tissue.

It is probable that small changes in a number of the fractions occur in many diseases. Thus the alpha 1, alpha 2 and gamma globulin fractions and also fibrinogen are increased during the acute phase of rheumatic fever.[16] They fall to normal as recovery ensues. The beta globulin fraction is not changed.

When subjected to electrophoresis, both mucoprotein and C-reactive protein migrate with the alpha globulin fraction.[2] C-reactive protein is absent from the blood of healthy persons; it is found in serum of patients in the acute stage of rheumatic fever and in other conditions.[1, 2, 27, 28] It is often the first laboratory test to return to normal in rheumatic fever, sometimes before any clinical signs of improvement are observed. Mucoprotein is normally present at a level of about 2.5 mg. of mucoprotein tyrosine per 100 ml. In our own experience, and in reports of others, it is increased during the acute phase of many conditions, particularly rheumatic fever, to levels as high as 16 mg. per 100 ml. Its return to normal level in rheumatic fever is relatively slow, and preliminary data suggest that it may reflect the progress of the disease better than other tests now being used.

It is of some interest that serum mucoprotein tyrosine is high in cancer of the liver.[10] Apparently it is below normal only in cirrhosis and in certain stages of the nephrotic syndrome.[13, 26]

The serum gamma globulin concentration rises in cirrhosis of the liver, cardiac failure, infections and in response to immunization. Serial determinations of gamma globulin concentration during infection may be of some value in following the course.[30]

REFERENCES

1. Adams, F. H., Kelley, V. C., Dwan, P. F., and Glick, D.: Mucolytic Enzyme Systems. XV. Response of the Serum Hyaluronidase Inhibitor and Mucoprotein to Adrenocorticotropic Hormone in Rheumatic States. Pediatrics, 7: 472, 1951.
2. Anderson, H. C., and McCarty, M.: Determination of C-Reactive Protein in the Blood as a Measure of the Activity of the Disease Process in Acute Rheumatic Fever. Am. J. Med., 8:445, 1950.
3. Bound, J. P., and Hackett, W. R.: Idiopathic Hypoproteinaemic Oedema and Aminoaciduria in an Infant. Arch. Dis. Childhood, 28:104, 1953.
4. Bruton, O. C.: Agammaglobulinemia. Pediatrics, 9:722, 1952.
5. Bruton, O. C., Apt. L., Gitlin, D., and Janeway, C. A.: Absence of Serum Gamma Globulin. Am. J. Dis. Child., 84:632, 1952.
6. Craig, J. M., Gitlin, D., and Jewett, T. C.: The Response of Lymph Nodes of Normal and Congenitally Agammaglobulinemic Children to Antigenic Stimu-

lation. Society for Pediatric Research, 24th Annual Meeting, Buck Hill Falls, Pa., May 5, 1954.

7. Darrow, D. C., and Cary, M. K.: Serum Albumin and Globulin of Newborn, Premature and Normal Infants. J. Pediat., 3:573, 1933.
8. Epstein, A. A.: A Contribution to the Study of the Chemistry of Blood Serum. J. Exper. Med., *16*:619, 1912.
9. Frick, P. G., and McQuarrie, I.: Congenital Afibrinogenemia. Pediatrics, *13*: 44, 1954.
10. Fried, C. T., and Henley, W. L.: Deficiency in Gamma Globulin with Edema and Hypoproteinemia. Pediatrics, *14*:59, 1954.
11. Gitlin, D., and Borges, W. H.: Studies on the Metabolism of Fibrinogen in Congenital Afibrinogenemia. Am. J. Dis. Child., *86*:646, 1953.
12. Good, R. A.: Agammaglobulinemia—An Experimental Study. Society for Pediatric Research, 24th Annual Meeting, Buck Hill Falls, Pa., May 5, 1954.
13. Greenspan, E. M., and Dreiling, D. A.: Serum Mucoprotein Level in Differentiation of Hepatogenic from Obstructive Jaundice. Arch. Int. Med., *91*:474, 1953.
14. Homburger, F., and Petermann, M. L.: Studies on Hypoproteinemia. II. Familial Idiopathic Dysproteinemia. J. Hematology, *4*:1085, 1949.
15. Imperatio, C.: Electrophoretic Studies of Serum Proteins of Well and Premature Infants. Lattonte, 22:449, 1951. Abstr. Am. J. Dis. Child., *83*:817, 1952.
16. Jackson, R. L., Kelly, G., Smith, E. K., Wang, P., and Routh, J. I.: Electrophoretic Analyses of Plasma or Serum Proteins of Rheumatic Fever Patients in Relation to Stages of Disease. Am. J. Dis. Child., *86*:403, 1953.
17. Janeway, C. A., Apt, L., and Gitlin, D.: Agammaglobulinemia. Tr. A. Am. Physicians, *66*:200, 1953.
18. Kagan, B. M.: The Protein Content of Normal Human Venous and Capillary Serum and Factors Affecting the Accuracy of Its Determination. J. Lab. & Clin. Med., 27:1457, 1942.
19. Kagan, B. M.: The Relationship between the Albumin-Globulin Ratio, Albumin, Globulin and Total Protein. Arch. Int. Med., *71*:157, 1943.
20. Kagan, B. M.: The Clinical Significance of the Serum Proteins. South. M. J., *36*: 234, 1943.
21. Kagan, B. M.: Hyperglobulinemia. Am. J. Med., *206*:309, 1943.
22. Kagan, B. M.: The Serum Proteins in Diseases of the Heart and Kidneys. Am. J. Clin. Path., *14*:327, 1944.
23. Kagan, B. M.: The Pediatric Significance of the Serum Proteins. Abraham Levinson Anniversary Volume. Studies in Pediatrics and Medical History. New York, Froben Press, 1949.
24. Kagan, B. M., Abt. A., and Morris, H.: Unpublished Studies from the Premature Nursery, Sarah Morris Hospital, Michael Reese Hospital, Chicago.
25. Keidon, S. E., McCarthy, K., and Haworth, J. C.: Fatal Generalized Vaccinia with Failure of Antibody Production and Absence of Serum Gamma Globulin. Arch. Dis. Childhood, *28*:110, 1953.
26. Kelley, V. C., and Panas, T. C.: The Nephrotic Syndrome in Children. II. Observations Concerning Acute Phase Reactions. J. Pediat., *41*:518, 1952.
27. Kelley, V. C., Adams, F. H., and Good, R. A.: Serum Mucoproteins in Patients with Rheumatic Fever. Pediatrics, *12*:607, 1953.
28. Kelley, V. C., Good, R. A., and McQuarrie, J.: Serum Mucoproteins in Children in Health and Disease, with Special Reference to Rheumatic Fever. Pediatrics, 5:824, 1950.
29. Laski, B., Korstak, A., and Hillman, D. A.: Cyclic Neutropaenia and Agammaglobulinaemia. Abstr., Society for Pediatric Research, 24th Annual Meeting, Buck Hill Falls, Pa., 1954.
30. Levin, M., Kaufman, H., and de la Huerza, J.: Gamma Globulin Studies in Tuberculosis in Children. Am. J. Dis. Child., *83*:26, 1952.
31. McMurray, Lucy-Gale, Roe, H., and Sweet, L. K.: Plasma Protein Studies on Normal Newborn and Premature Infants. Am. J. Dis. Child., *75*:265, 1948.

32. Metcoff, J., and Stare, F. J.: The Physiologic and Clinical Significance of Plasma Proteins and Protein Metabolites. New England J. Med., 236:26, 1947.
33. Norton, P., Kunz, H., and Pratt, E. L.: Electrophoretic Analysis of Serum Proteins in Premature Infants. Pediatrics, *10*:527, 1952.
34. Poyner-Wall, P., and Finch, E.: Protein Requirements of Infants. IV. Serum Protein Concentrations in Normal Full-Term Infants. Arch. Dis. Childhood, 25:129, 1953.
35. Rapoport, M., Rubin, M. D., and Chaffee, D.: Fractionation of Serum and Plasma Proteins by Salt Precipitation in Infants and Children. J. Clin. Investigation, 22:487, 1943.
36. Sanford, J. P., Favour, C. B., and Tribeman, M. S.: Absence of Serum Gamma Globulins in an Adult. New England J. Med., 250:1027, 1954.
37. Schick, B., and Greenbaum, J. W.: Edema with Hypoproteinemia Due to a Congenital Defect in Protein Formation. J. Pediat., 27:241, 1945.
38. Thompson, W. H., McQuarrie, I., and Bell, E. T.: Edema Associated with Hypogenesis of Serum Proteins and Atrophic Changes in the Liver with Studies of the Water and Mineral Exchanges. J. Pediat., 9:604, 1936.
39. Wyngaarden, J. B., Crawford, J. D., Chamberlin, H. R., and Lever, W. F.: Idiopathic Hypoproteinemia. Report of a Case of Transient Edema, Depression of Plasma Albumin and Gamma Globulin and Eosinophilia. Pediatrics, 9:729, 1952.
40. Young, W. F., Poyner-Wall, P., Humphreys, H. C., Finch, E., and Broodbent, I.: Protein Requirements of Infants. III. The Nutrition of Premature Infants. Arch. Dis. Childhood, 25:31, 1950.

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TRACHEOGRAPHY AND BRONCHOGRAPHY IN INFANTS AND CHILDREN

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The use of contrast studies in the younger age groups, when disease or anomaly of the tracheobronchial tree is suspected, has steadily increased as improvement in medical and surgical technique for care and treatment has demanded more accurate diagnosis. Our experience with studies of this kind extends over a period of nearly twenty years and many hundreds of examinations in patients ranging in age from early infancy to late childhood. One result has been the evolution of a technique which in our hands has allowed satisfactory studies in all but an occasional patient. At the outset it should be stated that tracheography and bronchography, though important, are only a part of the examination of the tracheobronchial tree and in most instances are done immediately after careful endoscopic inspection. Preliminary routine x-ray studies should always be done before special examinations of this kind are considered. Though safe in experienced hands, these are not techniques which should be attempted by those unfamiliar with endoscopic procedures despite their apparent simplicity.

INDICATIONS

On the whole, the indications for using these techniques are broad. In general, useful information, even of a negative nature, may be obtained when chronic bronchopulmonary changes are detected on physical or x-ray examinations and further differentiation is required for accurate diagnosis. When symptoms are chronic or recur frequently,

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the basic condition may be clarified by endoscopy and contrast studies. More specifically, the symptoms and signs which in most instances bring patients in the age group under discussion to this examination, as well as endoscopic examination, are as follows:

Cough

If the cause of a persistent cough cannot be determined by clinical studies including routine roentgenographic investigation, some underlying bronchopulmonary abnormality must be suspected. Many lesions may be associated with chronic cough that are not always easily detected or differentiated by routine x-ray studies. Among these are minimal bronchiectasis, bronchial stenosis, tracheal compression,[2] tracheomalacia, tracheo-esophageal fistula without atresia[1] and nonopaque nonobstructing foreign body. Such conditions can usually be demonstrated by endoscopy and contrast studies.

Stridor

Though the larynx is the most common site for the production of stridor in infancy, it is by no means the only one. When stridor is not explained by a laryngeal abnormality, further investigation of the tracheobronchial tree is indicated. Among the extralaryngeal causes of stridor are bronchial or tracheal stenosis or compression. The most severe stridor seen in our experience is that associated with the aortic arch anomalies. It is often expiratory as well as inspiratory and usually accompanied by dyspnea and retraction of the weaker areas of the chest wall. Cyanosis is sometimes noted when obstruction is severe.

Wheezing

This symptom is particularly important when localized to one area of the chest and may be constant or associated with recurrent infections. It is usually produced by any condition which results in narrowing of a bronchus, such as congenital stenosis or compression by tuberculous nodes, and is often confused with asthma.

Roentgenographic Signs

Localized emphysema or atelectasis in which the etiology is not apparent by routine radiologic methods and which is persistent for a matter of weeks needs further investigation and may be clarified by contrast studies. Recurrent peribronchial infiltration in the same pulmonary area may be caused by some abnormality which can only be

demonstrated by such studies. Recurring acute pneumonic processes in the same pulmonary area, apart from those occurring in bronchiectatic lobes, have been observed in instances when the right upper lobe bronchus arises directly from the trachea or a stenotic bronchus leads to a hypoplastic pulmonary area.

CONTRAINDICATIONS

Other than known sensitivity to the contrast material, there are no absolute contraindications to these procedures. However, it is not our practice to perform them in the presence of acute infection or during an acute asthmatic episode.

PREOPERATIVE PREPARATION

Hospital admission is recommended in all patients, since, among other reasons, any manipulation in the larynx may result in laryngeal edema which can develop slowly and not become manifest until some hours later. In infants who have severe obstructive symptoms no anesthesia is used, and no special preparation is required other than the omission of feeding and fluids for four hours prior to the procedure. In those infants receiving anesthesia, atropine in appropriate dosage is given forty-five minutes to one hour prior to anesthesia. Children over the age of one year receive one of the barbiturates, in addition to atropine, one hour before anesthesia time. When basal anesthesia with Avertin is contemplated, other sedation is omitted. We do not use morphine or scopolamine in our patients, since it has been our experience that these drugs have a depressing effect on respiration, which varies widely in degree and is unpredictable. In those patients who have large amounts of bronchial secretion postural drainage should be done before administration of the preoperative drugs when the cooperation of the patient can be obtained.

ANESTHESIA

With few exceptions, general anesthesia is used in carrying out these studies. The main reasons for not subjecting children to painful, frightening and sometimes physically traumatic experiences are too widely appreciated to require discussion. Apart from this, a more revealing examination can be carried out on an anesthetized patient in most instances than on one who is struggling and has to be restrained. Carefully administered general anesthesia has added little to the over-all risk, in our experience, and it has certainly made the procedure much easier for all concerned. When it is considered desirable to reduce the

amount of inhalation anesthesia, Avertin, in doses of 80 to 100 mg. per kilogram of body weight, may be administered by rectum. For patients who have had barbiturates or Avertin, induction is accomplished by vinyl ether (Vinethene) followed by drop ether, using the open mask method. In very young infants anesthesia is usually induced with drop ether alone.

The desirable stage of anesthesia for bronchography and tracheography is that in which the cough reflex is held in abeyance for two to four minutes. This allows sufficient time for injection of the contrast material, exposure of the films with the patient in suitable positions, and removal by suction of the contrast material. Ideally, the patient begins to cough during the course of the suctioning procedure and not before this time. Coughing during the course of injection almost always spreads the material into the alveoli and results in a "snowstorm" effect which obliterates the finer details of the tracheobronchial tree in most instances.

TECHNIQUE

Iodized poppy seed oil (Lipiodol) has been the contrast medium used in the vast majority of our patients. We have never observed any sensitivity to the oil or any untoward symptoms which could be attributed to chemical components contained in it. The oil is used at room temperature and spreads with great rapidity throughout the bronchial tree when injected. It is, therefore, necessary to use much less than one anticipates in most instances. The amount of oil required to outline the trachea in even a large infant is rarely over 2 cc. Any part of the tracheobronchial tree to which air passages are patent may be demonstrated. Thus the trachea or any part of the bronchial tree may be outlined as desired. Usually one of two procedures is performed: (1) a tracheogram in which the ideal objective is to inject just enough oil to demonstrate the trachea and beginnings of the main bronchi, or, (2) a bronchogram which demonstrates the bronchial tree of both sides and the trachea incidentally.

Tracheogram

Equipment. Illustrated in Figure 34 are the essential materials for this procedure: one 5- or 10-cc. syringe, a section of polyethylene tubing approximately 8 inches long, the iodized oil, a Jackson or Holinger infant anterior commissure laryngoscope with light cord and battery box, and a suitable suction tip. A good source of suction is absolutely necessary. Although not required in the examination at this point, it is wise to have an infant bronchoscope with attached oxygen insuffla-

tion tube at hand. Other equipment which should be present whenever any procedure is performed on an anesthetized patient are a plentiful supply of oxygen and a "cardiac arrest" kit for opening the chest should the need arise.

Procedure. 1. The patient is placed directly on an x-ray film casette in the supine position. The operator stands at the head of the table, and his assistant holds the patient's hips so that he may assist in turning the patient at the proper time.

2. The x-ray machine is pre-set for the lateral exposure, and the technician stands by, the exposure switch in hand.

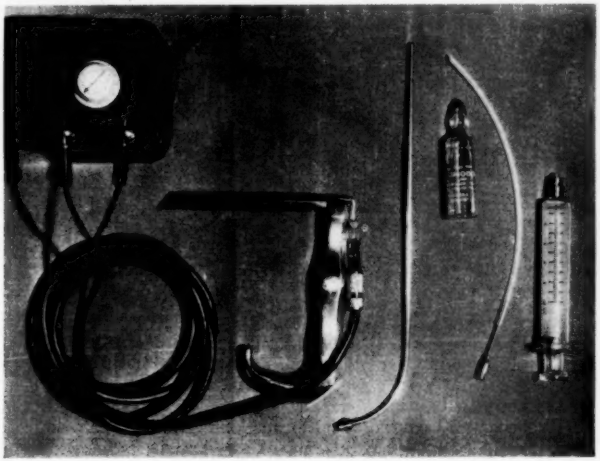

Fig. 34. Tracheogram tray, showing the essential materials. A somewhat longer polyethylene tube is used for bronchograms.

3. Using the anterior commissure laryngoscope in the left hand, the operator exposes the larynx and gently parts the cords with the tip of the instrument. Any secretion present in the larynx or trachea is removed by suction. The syringe containing the oil with the attached polyethylene tube is held in the right hand and the tubing passed, under direct vision, through the larynx about 2 cm. into the trachea. Depending upon the size of the patient, approximately 0.5 to 2 cc. of oil is injected. The tubing and laryngoscope are instantly removed and laid aside; should any obstruction to breathing be noted, an airway is inserted into the patient's mouth. The patient's hands are grasped by the operator and the arms brought into position above the head. The patient is turned into the lateral position and the film is immediately exposed. In most instances films are made on inspiration and expiration. When the lateral view (or views) is completed, a new casette is placed under the patient, who is now turned to the supine position, and an anteroposterior view is made.

4. The larynx is quickly exposed with the laryngoscope and the trachea cleared of contrast material by suctioning. Alternately, infants may be held upside down for a short while and "drained" of the oil. The entire procedure from start to finish usually takes about twenty to forty seconds. Figures 35, 36, 37 and 38 illustrate the essential steps. When actual x-ray exposures are being made, protective gloves and apron are worn. These were omitted in the photographs for the sake of clarity.

5. When the procedure is carried out on an anesthetized, dyspneic infant, speed

Fig. 35. Fig. 36.

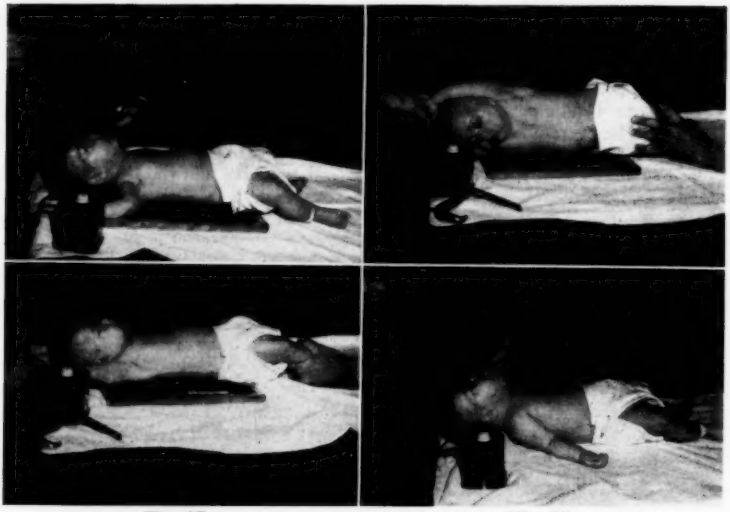

Fig. 37. Fig. 38.

Fig. 35. Tracheogram. Injecting oil with patient in supine position on x-ray casette.

Fig. 36. Tracheogram, lateral position. Inspiratory and expiratory films are usually made in this position. Protective gloves and apron are worn when actual exposures are made.

Fig. 37. Tracheogram. Patient in supine position for anteroposterior film.

Fig. 38. Tracheogram. Removal of oil by suctioning the trachea. Alternately, or if patient is not anesthetized, oil may be removed by "draining."

is of the essence if good films are to be made and the patient disturbed as little as possible. It is necessary to have two extra assistants under these circumstances, one to hold the patient's head and the other to change the film casettes. Unanesthetized patients are almost always "drained" of oil by simply holding them upside down, since this disturbs them far less than exposing the larynx and suctioning the trachea.

Bronchogram

This procedure may be done at any age, though, in our experience, it is performed far more often after the age of five than before it and in infancy much less frequently than a tracheogram. Any part of the tracheobronchial tree of either or both sides may be demonstrated at one sitting. *Good results depend upon the proper stage of anesthesia and the rapid and orderly completion of the procedure.*

Equipment. With one important exception, the equipment is essentially the same as that used for the tracheogram. In many instances a plastic intratracheal tube is inserted to maintain a clear airway during the course of the examination. In some instances when respiratory function is impaired, a metal Y tube is attached to the external end

of the intratracheal tube, and through one limb of the Y, oxygen may be administered. Under such circumstances the polyethylene tube through which the oil is instilled is inserted first into the trachea to about the level of the carina. A plastic intratracheal tube of suitable size is then introduced into the trachea, after which the Y tube is attached. With this arrangement it is possible to keep the patient well oxygenated and, if necessary, administer anesthesia. The polyethylene tube may be

Fig. 39. Bronchogram, showing the introduction of the polyethylene catheter through the plastic intratracheal tube for purpose of injecting iodized oil into the tracheobronchial tree.

moved in or out as necessary. When it is not considered necessary to continue anesthesia or administer oxygen, the polyethylene catheter may be passed directly through the intratracheal tube, provided sufficient lumen is left for respiratory exchange (Fig. 39).

Procedure. 1. The filling of the tracheobronchial tree is done under fluoroscopic guidance and observation by the roentgenologist. The patient is placed in the supine position on the x-ray table and the fluoroscopic screen moved over the chest. The polyethylene catheter is inserted at this time if the arrangement described earlier, including the intratracheal tube, has not already been introduced.

2. Under fluoroscopic observation enough Lipiodol is injected to fill the catheter and demonstrate its location. It is then turned in such a way that it enters the right main bronchus. The patient is turned slightly to the right side, and sufficient oil is injected to fill the bronchial divisions on this side exclusive of the middle lobe. The polyethylene catheter is then withdrawn to the carina, turned slightly, and passed into the left main bronchus. The bronchial divisions on this side are then filled, and the syringe containing the oil is disconnected and the catheter left in situ. The fluoroscopic screen is moved away, and the patient is turned immediately into the prone position so that the oil will enter the middle lobe, and placed on a casette.

3. The patient's arms are in position over the head, with the head turned to one side. An exposure is then made in the postero-anterior position. The casette is replaced and one postero-anterior oblique view with the right side against the casette

Fig. 40. Fig. 41.

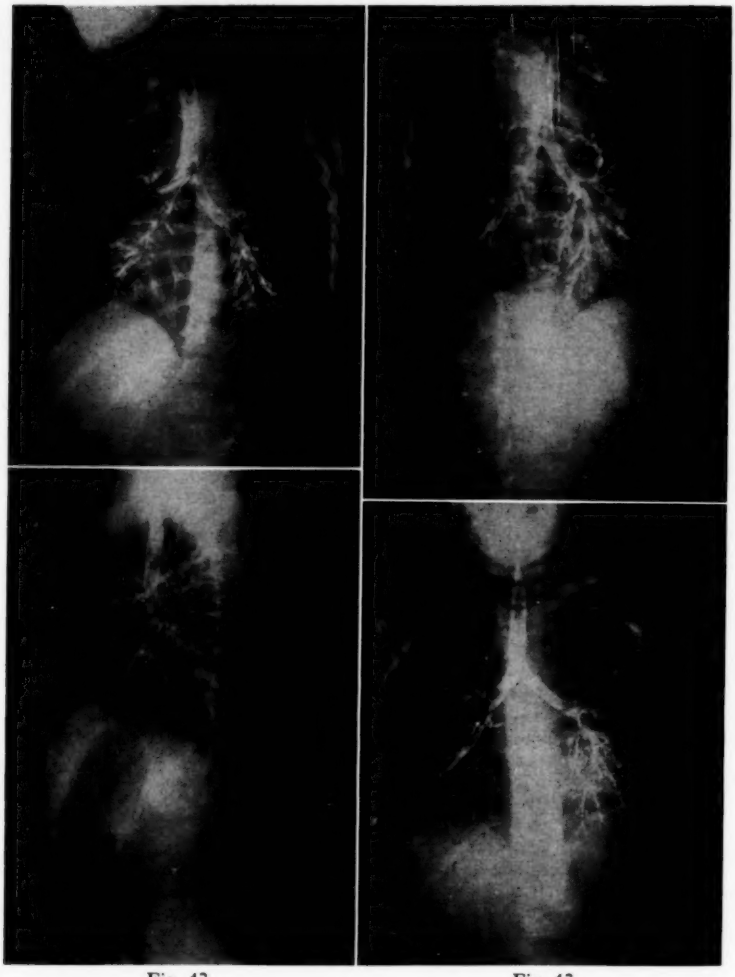

Fig. 42. Fig. 43.

Fig. 40. Bronchogram, left oblique view.
Fig. 41. Bronchogram, right oblique view.
Fig. 42. Bronchogram, lateral view.
Fig. 43. Bronchogram, anteroposterior view. The postero-anterior view was omitted from this series, but is usually taken.

and the body at an angle of 30 degrees to the table is made. A similar postero-anterior oblique view is then made with the left side next to the casette and the body at an angle of about 30 degrees to the table. A straight lateral view is made and the patient is then turned over to the supine position, and an antero-posterior view is exposed.

In all, five views are made which, when the filling is successful, will give an excellent view of all the bronchial divisions (Figs. 40, 41, 42 and 43).

4. Suctioning of the oil from the tracheobronchial tree is then carefully carried out. The mouth and nose are cleaned by catheter suction, and the tubing is withdrawn from the trachea. An airway is placed in the mouth, and the patient is then returned to the prone position with one shoulder elevated slightly and the head extended. The procedure can usually be accomplished in two to three minutes. When only a limited portion of the tracheobronchial tree is to be demonstrated, a much shorter time is required.

POSTOPERATIVE CARE

When the patient has been cleared of the oil, either by suctioning or "draining," no special postoperative care is required other than that given to any recently anesthetized patient. Almost without exception, the patient is discharged from the hospital the following day unless further investigation or operative procedures are contemplated. Elevation of temperature, vomiting or other untoward symptoms have rarely been observed. Laryngeal edema of significant degree has never occurred in our experience, and edema even of recognizable degree is rare indeed.

DISCUSSION

In carrying out the tracheogram technique the first films are exposed in the lateral position, since serious tracheal compression is almost always in the anteroposterior diameter, and this position has proved to be the most revealing one when tracheal lesions are suspected (Figs. 44, 45, 46 and 47). Whenever feasible, films should be exposed for both inspiration and expiration, since an erroneous conception of the relationship of the walls of the trachea and the degree of compression on collapse may be obtained if a film made in only one phase of the respiratory cycle is seen. In infants one should bear in mind that, in comparison with older children, very little oil is needed because of the small size of the airway and the rapid dispersion of the oil. In the newborn 0.5 cc. or less is adequate to demonstrate the trachea, and 1 cc. will often give enough filling of the tracheobronchial tree for a satisfactory bronchogram. (In some instances when the airway is stenosed, the addition of 1 cc. or more of oil to the already small passage may momentarily accentuate the patient's respiratory distress and produce changes that are more apparent than real.) In some instances in which we have been able to see and visually demonstrate the changes by endoscopy, but not by the routine tracheogram technique, the serial tracheogram has proved useful. With special equipment it is possible to expose fifteen to twenty films with the patient in the lateral position, during several respiratory cycles. Unfortunately, this equipment is not standardized or widely available at present.

Fig. 44.

Fig. 45.

Fig. 46.

Fig. 47.

Fig. 44. Tracheogram. Patient is an infant with marked constriction of trachea and esophagus due to vascular ring.

Fig. 45. Tracheogram. Lateral view of patient shown in Figure 44.

Fig. 46. Combined tracheogram-esophagogram to show tracheo-esophageal relationship. Note defect in esophagus below carina caused by aberrant vessel.

Fig. 47. Lateral view of patient in Figure 46 showing normal tracheal lumen and same defect in esophagus seen in Figure 46.

Each of the techniques described is useful not only for diagnostic purposes, but for evaluating the results of medical and surgical therapy as well. In patients with bronchiectasis or other chronic pulmonary diseases who are under medical supervision, carefully done contrast studies at suitable intervals will often give valuable information as to the effectiveness of therapy. Postoperative follow-up studies on patients who have had surgical intervention will give pertinent information useful as a matter of record or as a guide in their future care.

SUMMARY

Contrast studies of the various parts of the tracheobronchial tree, preferably with the patient anesthetized, may be done at any age and are often of critical diagnostic value. The symptoms indicating the need for these studies are chiefly cough, stridor, wheezing or roentgenographic signs resulting from bronchial or tracheal obstruction and other changes of a chronic nature. The technique, though simple, presupposes a knowledge of endoscopic procedures and, to be successful, requires the cooperative efforts of the anesthetist, endoscopist and roentgenologist. When patients are handled with skill and dispatch, the procedures are safe and the results rewarding.

REFERENCES

1. Ferguson, C. F.: Congenital Tracheoesophageal Fistula not Associated with Atresia of the Esophagus. Laryngosocope, *61*:718, 1951.
2. Gross, R. E., and Neuhauser, E. B. D.: Compression of the Trachea or Esophagus by Vascular Anomalies. Surgical Therapy in 40 Cases. Pediatrics, *7*:69, 1951.

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ELECTROENCEPHALOGRAPHY IN CHILDREN

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Electroencephalography is the technique of recording and the science and art of interpreting the electrical activity of the brain. It is entirely proper that an article on electroencephalography should deal specifically with the electroencephalograms of children, because electrical activity of the brain in childhood is different from that in adult life. Certain types of disorder which show clearly in the electroencephalograms of children are rarely encountered in adults, and vice versa. The great majority of epileptic disorders begin in childhood, and the electroencephalogram is particularly useful for the diagnosis and localization of epileptic disorders of children. For these reasons the electroencephalogram has special interest and value for the pediatrician.

In the twenty-five years since Hans Berger of Jena first demonstrated that the electrical activity of the brain can be recorded as a more or less rhythmic pattern through the unopened skull, electroencephalography has developed into a generally accepted aid to the diagnosis and localization of brain disorders. The electrodes usually used in electroencaphalography are fastened to the scalp. Sometimes recordings are made from the nasopharynx or the tympanic membrane, and at operations neurosurgeons often record from electrodes applied directly to the surface of the brain or inserted into the substance of the brain. Variously located electrodes on the surface of the head permit the registration of activity from subjacent cortical areas.

The recording of an electroencephalogram is much more difficult and more time consuming than the recording of an electrocardiogram, but it is also more rewarding, for it has a wide variety of correlations with clinical signs and symptoms. An abnormal electroencephalogram

is usually obtained in cases in which the cortex is the site of acute damage or severe physiologic disorder—for example, anoxia, hypoglycemia, irritation or inflammation. Absent or dead neurons and scars, tumors, blood clots and abscesses do not themselves give off electrical signals, but it is the injured neurons surrounding the pathologic processes that indicate trouble by developing abnormal electrical rhythms. They may indicate disorder by beating with an abnormally fast or slow rhythm or by developing irregular, paroxysmal discharges. Neurons that show abnormal electrical activity are not necessarily structurally damaged; almost all types of electroencephalographic abnormality are reversible. Electrical foci of abnormality may disappear spontaneously or migrate from one location to another. The electroencephalogram seldom points to an etiologic diagnosis; it reveals the type of abnormal discharge and its temporal and spatial relationships. The electroencephalogram has generally good correlations with age and states of consciousness (alert, drowsy, asleep and comatose). Irritative and depressive types of injury to cortical neurons and clinical epilepsy are usually associated with electroencephalographic abnormality.

CHANGES WITH AGE

Age changes are so great that an experienced electroencephalographer can estimate the age of a child from the electroencephalogram. This is particularly true when the interpretation is based on a recording in which the various stages of drowsiness and sleep are shown. In order to get the maximum amount of clinical information from the recording the patient should be examined not only while awake, but also during spontaneous or drug-induced sleep. Studies carried out on the same patient during spontaneous and Seconal-induced sleep have shown that the latter is as good as normal sleep for bringing out seizure discharges and other abnormalities.

Recording in both the waking state and sleep is important, but in pediatric electroencephalography sleep is particularly important because most children do not keep still enough to obviate movement and muscle artefacts. Although localized and diffuse depressive reactions to injury usually show well in the waking state, irritative reactions and epileptic disorders are usually more evident during sleep. If patients with a history of epileptic seizures are studied only while awake, the incidence of clearly evident seizure discharges is only about 35 per cent; but if they are studied during sleep, 85 per cent will be found to have seizure activity in their electroencephalograms.[2] Epilepsy originating in the anterior portion of the temporal lobe is usually non-

evident in the waking electroencephalogram, but usually shows clearly during sleep. Electroencephalographic evidence of diencephalic epilepsy is almost never seen except in sleep.

THE NORMAL ELECTROENCEPHALOGRAM DURING THE FIRST TWO DECADES

Although the course of development of electroencephalographic patterns varies to a certain degree from one person to another, certain trends are evident in normal infants and children, and the specific changes can be stated in terms of upper and lower limits in relation to age. The baby during the first month of life shows low voltage, irregular slow waves as a basic background while awake. He has no special patterns of drowsiness, and the stages of sleep are only poorly defined. During the first three months the patterns are so irregular and so poorly developed that the clinical value of the electroencephalogram during that age period is somewhat limited: in some cases, however, seizure discharges or other abnormalities are evident in the first three months and are highly significant.

As the baby becomes older, its brain waves become faster and of higher voltage. Typical high voltage, slow waves of drowsiness first appear at two to three months, and the characteristic biparietal humps of light sleep appear at about six months. Although the humps remain characteristic of light sleep throughout most of adult life, they are the highest in voltage and most conspicuous from three to ten years; after fifty years they usually disappear. Another characteristic feature of light sleep, namely, bursts of 14-per-second activity (spindles) which are strongest in the parietal areas, develops at two to three months and remains through most of life, but tends to diminish in old age.

The general trend of development from early infancy to adulthood is toward speeding up of activity; voltage rises through the first two years and tends to decline after the twelfth year. Many features, especially the high voltage, slow waves of drowsiness that occur in young children, would be abnormal in the late teens. The regular adult pattern usually develops by twenty years and changes little from that age to forty. Deep sleep obliterates the characteristic patterns that differentiate the various age groups, and it reduces seizure patterns, so that it is not generally used in the routine electroencephalographic examination.

THE ABNORMAL ELECTROENCEPHALOGRAM

We cannot give statistics on all clinical entities in which the electroencephalogram is useful, but we shall indicate how it is useful in those conditions in which the pediatrician is likely to be most interested.

Congenital Anomalies

At present there are no electroencephalographic abnormalities that are recognized as specifically diagnostic for any particular congenital anomaly of the brain. The major deviations from normal structural development can be diagnosed by inspection or by x-ray films without air replacement; the electroencephalograms in such cases may or may not be abnormal. Porencephaly cannot be diagnosed by electroencephalograms, but may be suspected if a slow wave focus or localized seizure discharge is found.

Encephalitis

In the acute phase of encephalitis diffuse, slow activity is usual. The degree of slowing runs roughly parallel with the severity of the encephalitis. In the postacute phase the electroencephalographic changes usually disappear more gradually than the clinical symptoms. Slow activity may disappear completely or a localized slow wave focus may persist, or after the most severe slowing has cleared up spikes may appear. These spikes may remain asymptomatic, but they are a sign that a seizure process is present which may flare up and produce clinically evident seizures. In most cases in which encephalitis leaves permanent after-effects in children, electroencephalographic changes also remain. The electroencephalogram is a fairly reliable guide in the follow-up of children with proved and suspected encephalitis.[5]

Subdural Hematoma

The only reliable way to prove or rule out a subdural hematoma is by tapping the subdural space. Because the technique is so simple and easy in infants, it certainly should be used on any suspicion that a subdural hematoma may be present. A reduction of voltage over a subdural hematoma is common, and in some cases a slow wave focus occurs, but these abnormalities do not occur regularly enough to be of much practical value and should not replace direct investigation.

Brain Tumors

Brain tumors in children are commonly subtentorial, and the electroencephalogram is not able to localize subtentorial lesions accurately. Therefore electroencephalography is not particularly useful for the diagnosis and localization of brain tumors in childhood, but cerebellar and large subtentorial tumors, particularly in children, often produce slow wave activity in one or both occipital areas. When the tumor is supratentorial and involves the outer surface of the cerebral hemispheres,

there is approximately an 80 per cent chance that it will produce a slow wave focus which will accurately indicate the location of the tumor.

Electroencephalogram in Epilepsy

Scientifically speaking, epilepsy is a disorder of energy release within the central nervous system, characterized by an excessive release of energy per cubic centimeter of brain per second. Persons with epilepsy have many more subclinical seizures (i.e., seizure discharges without clinical manifestations) than clinical seizures. It is this fact that makes possible the electroencephalographic detection of epileptic disorders in the majority of persons with epilepsy. Epilepsy which is subclinical may seem paradoxical, but from a medical point of view it is a natural and reasonable concept. Any disease process which is not accompanied by manifest clinical symptoms can be said to be *subclinical*. Subclinical epilepsy is no more paradoxical than subclinical gallbladder disease or subclinical diabetes. One of the chief values of the electroencephalogram is that it gives evidence of epileptic disorders when the clinical symptoms are insufficient for a positive diagnosis.

Different types of epilepsy tend to occur at different ages, probably because a young brain's reaction to injury is different from that of an old brain, and probably also because different types of injury tend to occur at different ages. Generalized epileptic disorders are common in children and focal disorders in adults, but focal epilepsy can occur at all ages. Some disorders that at the onset look benign, when viewed over a period of years are seen to be exceedingly malignant. On the other hand, some disorders that appear malignant at the onset tend to clear up with increasing age. An understanding of the prognosis of various types of epilepsy is important, for otherwise therapy might be undertaken which is either too radical or else not sufficient, considering the gravity of the prognosis. *Epilepsy* is too broad a diagnosis for practical clinical purposes. The dichotomy symptomatic-idiopathic epilepsy has lost almost all meaning as increased knowledge has developed regarding subclassification of the epilepsies.

Hypsarhythmia. A type of seizure activity which is more or less continuous and always generalized and which is found only in very young children has been called hypsarhythmia.[3] The clinical symptoms are quivering attacks, brief spasms, and sudden jerks of the head or the entire body. These symptoms often seem rather benign to the parents, but this is the most serious form of epilepsy. Ten per cent of babies with hypsarhythmia die during the first few years of life; 80 per cent of infants with such disorder grow up with severe intellectual impairment; and only 10 per cent recover from this condition.

The etiology of this devastating form of epilepsy is in most cases

undetermined. Various kinds of insults produce it; anoxia neonatorum, encephalitis and birth trauma appear to be important causes. In a small percentage of cases clinical and electroencephalographic normality has been obtained after intensive antibiotic medication.[6]

The electroencephalographic pattern of hypsarhythmia is characterized by high voltage, slow "mountainous" waves, mixed with single or multiple high voltage spikes.

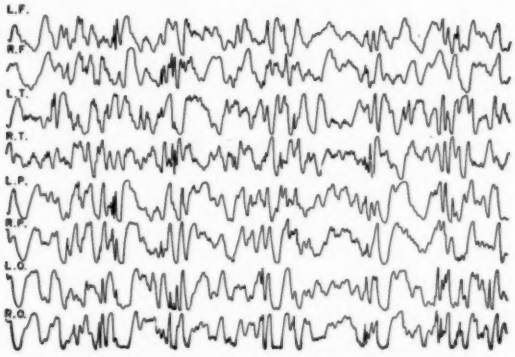

Fig. 48. Electroencephalograms in hypsarhythmia.

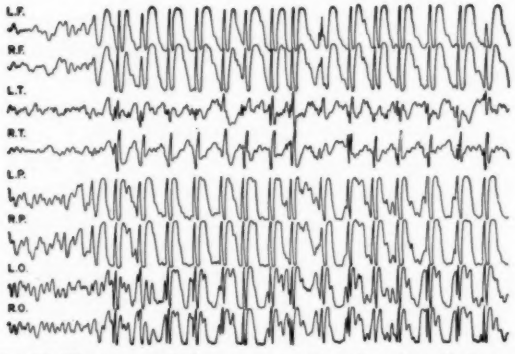

Fig. 49. Electroencephalograms in petit mal variant discharge.

Petit Mal Variant. The next most malignant epilepsy occurs chiefly between the ages of two and six years; because of its resemblance to the electroencephalographic pattern that occurs in immediate temporal association with petit mal seizures, it has been referred to as ***petit mal variant***. The characteristic pattern of petit mal variant consists of an alternating high voltage spike and slow wave which repeats twice a second. Children with this type of disorder often have falling spells and tend to have frequent and severe generalized convulsions. Intellectual

defects are also common in this group. Neurologic signs indicating organic damage are frequent.

As children with hypsarhythmia or petit mal variant grow older, their generalized dysrhythmias usually disappear and they develop spike foci.

Petit Mal. Petit mal activity is the pattern which is more or less characteristic of the child of school age; it is most often encountered in the age group between five and fifteen years. It rarely occurs in younger children or adults. *Petit mal* as used here is not just any mild type of seizure, but a specific type of seizure. It may occur alone or in combination with grand mal. The clinical seizures usually last only a

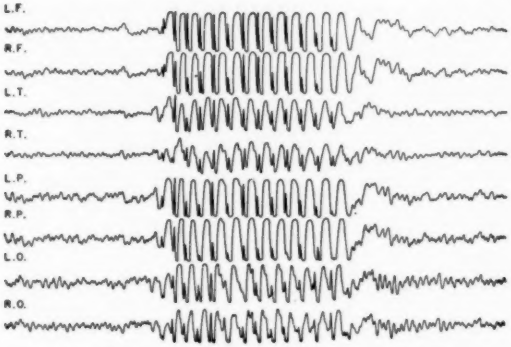

Fig. 50. Electroencephalograms in petit mal discharge.

few seconds and consist of one or more of the following: loss of consciousness usually without loss of posture, staring, and rhythmic blinking and nodding of the head. Petit mal status with long periods of confusion occurs in severe cases and is often hard to distinguish from psychomotor seizures.

The typical pattern consists of an alternating spike and wave which repeats three times a second. This form of epilepsy is usually benign and is not associated with intellectual impairment or evidence of brain damage and tends to disappear in late adolescence. Usually the clinical seizures cease before the seizure discharges entirely disappear.

Tridione and Paradione are the best drugs for the treatment of petit mal, but they may activate convulsions, particularly in cases in which a grand mal component (multiple spikes) is evident in the electroencephalogram. These drugs tend not only to control attacks, but also to normalize the electroencephalogram.

Focal Epilepsy. The first two epileptic conditions that have been mentioned occur exclusively in childhood, and the third, namely, petit mal, is rare after the second decade; these are all generalized types of

disorder. Localized epileptic discharges are most common in adults, but are not rare in children. An interesting feature of epileptic foci in childhood is that they are not fixed; they tend to migrate, and this migratory tendency is evident not only in so-called idiopathic epilepsy, but also in so-called symptomatic epilepsy, although the structural lesion does not change and the neurologic deficits remain fairly constant. If an

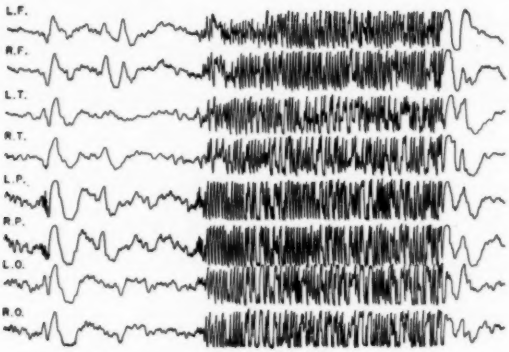

Fig. 51. Electroencephalograms in grand mal discharge.

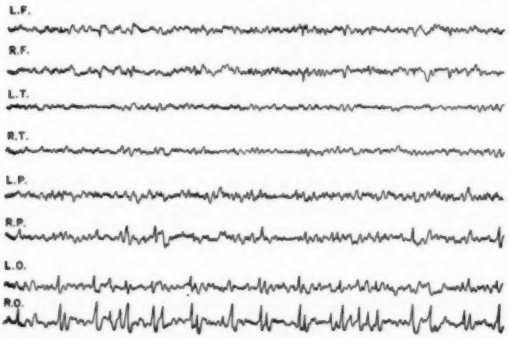

Fig. 52. Electroencephalograms in occipital focus of seizure activity.

epileptic focus is found in a child below school age, it is most likely to be in the occipital area. Focal epileptic disorder in the occipital lobe is often associated with convulsions, but eye signs, visual defects and atypical seizures (often described as "not epileptic") consisting of dizzy spells, vomiting attacks and staring spells are also frequently encountered (Fig. 52).

By nine years of age 48 per cent of patients who previously had occipital lobe foci cease to have seizures, lose their focus and develop a normal electroencephalogram.[4] Foci that do not clear up, either con-

tinue in the occipital lobe or migrate to some other region, usually the midtemporal.

Midtemporal Epileptic Focus. A focus of seizure activity in the midtemporal area is commonest in the ages between seven and ten years. Such a focus is usually associated with major convulsions, but submaximal discharges in this area may manifest themselves in focal seizures usually involving one side of the face and occasionally one arm. Fear, either in the form of attacks or as continual fearfulness, though not exceedingly common, is much commoner in association with a midtemporal focus than with any other type of epilepsy. Seizure discharges

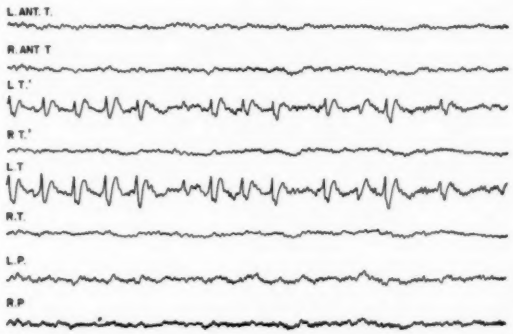

Fig. 53. Electroencephalograms in midtemporal focus of seizure activity.

in the midtemporal area tend to subside or migrate to some other area with increasing age. Fifty per cent of patients who previously had a midtemporal lobe focus when studied after fifteen years of age were found to have normal electroencephalograms and were seizure-free.[4] When the midtemporal focus does not clear up, it continues in the same place or is transformed into 14- and 6-per-second positive spikes or migrates into the anterior temporal region.

Epilepsy with 14- and 6-per-Second Positive Spikes. This type of disorder is most common in adolescents and young adults, but is not rare in the younger age groups.

These 14- and 6-per-second positive spikes appear in light sleep, and are usually seen in the temporal and occipital areas simultaneously. They are either unilateral or bilateral; when they are bilateral, the discharge usually occurs independently in one hemisphere and then in the other. The clinical correlates of this type of discharge are attacks of dizziness, nonorthostatic syncope, episodes of pain, attacks of gastrointestinal or vegetative symptoms and attacks of rage;[4] the pediatrician will find it masquerading in many forms. Convulsions occur in approximately 50 per cent of children with this type of dis-

charge. In spite of the wide range of symptoms that are associated with this condition the clinical story is sufficiently characteristic so that the physician is often able to predict that 14- and 6-per-second positive spikes will be found in the electroencephalogram. What is often referred to as migraine in childhood commonly turns out to be 14- and 6-per-second positive spike epilepsy. So also, recurring abdominal pains in children and certain behavior disorders can be diagnosed with the help of the electroencephalogram as belonging to this type of epilepsy and can often be controlled with anticonvulsant medication, thus obviating long psychiatric treatment.

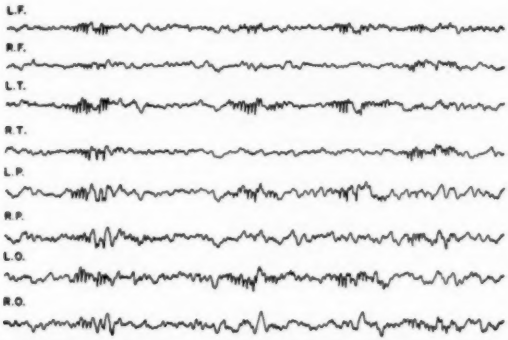

Fig. 54. Electroencephalograms with 14- and 6-per-second positive spikes.

In the few patients with this pattern who have come to surgery,[7] and in some cases in which a gross lesion was localized by pneumograms, the lesion was usually found to involve the thalamic or hypothalamic areas. This, together with the character of the associated symptoms, has suggested that 14- and 6-per-second positive spikes are evidence of thalamic or hypothalamic epilepsy. Fortunately this is a relatively benign type of epilepsy, and like other types of epilepsy it tends to subside with increasing age.

Anterior Temporal Lobe Foci. Anterior temporal lobe foci are found frequently in adults, but are not rare in childhood. Seizures produced by these foci are usually trancelike attacks or episodes of confusion with purposeless but reasonably well coordinated movements; these attacks are usually called *psychomotor* seizures.

Without benefit of electroencephalography mild attacks of this type can easily be confused with petit mal; because they are frequently followed by a convulsion, they are often overlooked by parents, friends and even the doctor. An electroencephalogram without sleep will show an anterior temporal lobe focus in only one third of the cases that have

clinical evidence of psychomotor epilepsy. If sleep recordings are made, an anterior temporal focus is found in 80 per cent of the cases. Focal seizure discharges may be present in one anterior temporal lobe, or they may come from both temporal lobes independently. The anterior temporal focus does not tend to disappear or to migrate to other areas of the brain.

If drug treatment cannot prevent the seizures and the patient becomes totally disabled and the focus is predominantly unilateral, surgery in the form of amputation of the tip of the temporal lobe, including a

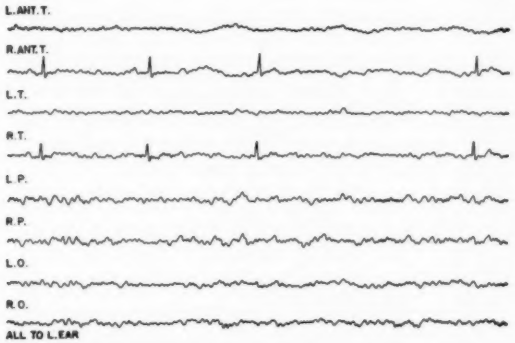

Fig. 55. Electroencephalograms in anterior temporal focus of seizure activity—psychomotor type of focus.

part of the mesial surface, may become indicated. Such surgery will seldom, if ever, be necessary in childhood, and should be done only under the guidance of electroencephalographic recordings from the exposed brain.

Cerebral Palsy

Convulsions are a common complication of cerebral palsy, but seizure discharges may not manifest themselves until some time late in the first year of life, and electroencephalographic evidence of the seizure process is usually greatest around the fifth year, subsiding somewhat thereafter. If seizure discharges are present, they create some presumption that clinical seizures may occur, but the epileptic process may remain subclinical and subside without producing clinical manifestations. Follow-up studies on these children also show that spike foci often tend to disappear in the second decade even when no improvement of the neurologic deficit occurs. When the electroencephalographic abnormalities disappear, the clinical seizures usually stop; so an improving electroencephalogram is a good prognostic sign.

Mental Deficiency

There is no good correlation between the electroencephalogram and intelligence. Exceedingly feeble-minded children may have normal electroencephalograms. However, work now in progress at the Dixon State Hospital under the direction of Dr. Alberto Fois indicates that in most cases of so-called undifferentiated feeble-mindedness, in most postencephalitic states and in such conditions as phenylpyruvic oligophrenia there are significant changes in the electroencephalogram.

Behavior Disorders

To order an electroencephalogram on every child who has a behavior disorder would not be practicable. Common sense, parental understanding and the physician's clinical judgment should help to screen out those cases in which electroencephalography is entirely unnecessary. However, as time goes on electroencephalography is likely to be used more and more as a general screening test. Rage attacks which fall outside the bounds of normal childish behavior may be related to 14- and 6-per-second positive spikes. Many children with mild complaints of dizziness and visceral disturbances will be found to have 14- and 6-per-second positive spikes. Midtemporal lobe epilepsy is so common among school age children that fearfulness which is considered functional may turn out in some cases to be based upon an epileptic disorder in the midtemporal area. In many cases it is difficult to differentiate between the classical types of schizophrenia and psychomotor epilepsy without an electroencephalogram.

Febrile Convulsions

Febrile convulsions are treated separately in this discussion because they will present themselves as an important clinical entity to the physician in pediatric practice. Children with febrile convulsions may be grouped into those with the seizures precipitated by the fever itself and those in which the fever is incidental to the convulsion. Studies of patients belonging to the latter group reveal that these are contaminated with children whose electroencephalograms indicate that they are suffering from an acute encephalitis, and later electroencephalographic studies will reveal that the child is following either a course of improvement or increasing abnormality with the appearance of seizure discharges. The prognosis depends on which course the child follows.

In those patients with true febrile convulsions, when seizures occur repeatedly with febrile illnesses and not at other times, the electroencephalogram is usually normal. This is the most benign type of

epilepsy. These seizures usually cease by the age of five years and are not usually followed in later life by epileptic manifestations. Strangely enough, febrile convulsions are the most inheritable form of epilepsy. In approximately 25 per cent of children with febrile convulsions there is a family history of the same type of disorder.[1]

Breath-Holding Spells

The electroencephalograms of children with breath-holding spells are in general as normal as those of random control groups; this suggests that breath-holding spells are not a type of epilepsy.[1, 2]

REFERENCES

1. Bridge, E. M., Kajdi, L., and Livingston, S.: A 15 Year Study of Epilepsy in Children. A. Research Nerv. & Ment. Dis., Proc., *29*:451, 1947.
2. Gibbs, E. L., and Gibbs, F. A.: Atlas of Electroencephalography. Cambridge, Mass., Addison-Wesley Press, Inc., 1950 (Vol. I) and 1952 (Vol. II).
3. Gibbs, E. L., Fleming, Marilyn M., and Gibbs, F. A.: Diagnosis and Prognosis of Hypsarhythmia and Infantile Spasms. Pediatrics, *13*:66, 1954.
4. Gibbs, F. A.: Diagnosis and Prognosis of Different Types of Epilepsy. Cincinnati J. Med., to be published.
5. Grossman, H. J, and Gibbs, E. L.: Clinical and Electroencephalographic Studies of Patients Developing Encephalitis Following Measles. Read before the American Academy of Neurology, April 9, 1953.
6. Stamps, F. W., Gibbs, E. L., and Haase, E.: Epileptic Patients Treated with Aureomycin. Dis. Nerv. System, *12*:227, 1951.
7. Stephenson, W. A.: Intracranial Neoplasm Associated with 14 and 6 per Second Positive Spikes. J. Neurol., Neurosurg. & Psychiat., *1*:372, 1951.

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THE VALUE AND CLINICAL APPLICATION OF DETERMINING BACTERIAL SENSITIVITY TO ANTIBIOTICS

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The age of "wonder drugs" has brought to clinical medicine more than "miraculous" cures of some of the infectious diseases. It has brought its own aura of clinical diagnostic problems, clinical laboratory tests, clinical complications of treatment, and also profuse and confusing scientific and advertising literature. This clinic will attempt to clarify one of these aspects, namely, the laboratory evaluation of the antibacterial effects of the antibiotics, and the interpretation of the results for their application to the care of patients.

With the advent of sulfonamides and penicillin, in vitro techniques were originated and subsequently applied to all the other antibiotics to measure the inhibition of the growth of bacteria in artificial media. Such studies became known as sensitivity tests. When bacterial growth was suppressed by a low concentration of an antibiotic, the strain was said to be "sensitive" to that agent. The effect of different antibiotics upon sensitive strains might be *bactericidal*, that is, killing the bacteria that were exposed to it, or *bacteriostatic*, that is, preventing the growth of bacteria without destroying their ability to proliferate when the antibiotic was removed. Actually, all the common antibiotics are both bacteriostatic and bactericidal, but to different degrees under a given set of conditions.

Modifications of the sensitivity test were proposed to measure the rapidity of bacterial death or the time of their recovery after exposure

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to different concentrations of an antibiotic. Other modifications of the sensitivity test were suggested to simulate more closely physiologic conditions of infection, such as the effect of proteins in binding an antibiotic and the influence of cellular constituents upon the inhibitory activity of the agents. Also, biologic antagonists to the effects of an antibiotic and antibiotic-destroying substances were identified. Finally, the sensitivity test has been adapted to make specific biochemical and genetic observations of the effects of antibiotics upon bacterial metabolism, and cell division.

A sensitivity test, therefore, might measure any of a number of effects of an antibiotic upon bacterial growth and always yields results that need not apply to any conditions except those under which the test was performed, many of which can be altered at will. In the practical guidance of therapy, only one or two aspects of sensitivity tests have clinical importance.

Physicians have been profoundly influenced in the treatment of their patients by the addition of sensitivity tests to their armamentarium. The availability of the procedure has lent an appropriately scientific attitude of quantitation and specificity to the treatment of clinical infections. However, in the practical utilization of bacterial sensitivity tests the usefulness and limitations of the procedures have not been appreciated uniformly. Two typical and opposite reactions have been observed: (1) the belief that every strain of microorganism isolated from a patient suffering from an infection should be studied for inhibition in vitro by several different antibiotics, and the treatment be dictated strictly by the results of the sensitivity tests; (2) the belief that one need not do any antibiotic sensitivity testing, and often not even obtain cultures unless the patient fails to respond to treatment with an antibiotic. Neither of these extremes of opinion yields the optimum benefit to be gained from sensitivity tests in the treatment of infections.

One of the effects of the antibiotic era upon the hospital laboratory for clinical bacteriology has been a deluge of requests for bacterial sensitivity tests, often requiring the employment of additional technicians or the setting aside of other work on certain days. Another consequence has been a diminishing interest on the part of clinicians in the methods of bacterial isolation and the recognition of species and types of microorganisms. The result has been a tendency toward less careful identification of bacteria, and the seeming necessity to acquire for routine use a rapid and easy method to estimate roughly the sensitivity or resistance of a bacterial strain to the satisfaction of the clinician. It is apparent to anyone who sees patients in different hospitals that the results of routine sensitivity testing may have little comparative value. Some technical aspects in the performance of the sensitivity test

are of great importance in determining the result, and certain of these aspects will be discussed.

BACTERIAL AND ANTIBIOTIC SPECTRUMS

Out of careful laboratory observations upon the ability of a single antibiotic to inhibit different families of bacteria grew the concept of the "bacterial spectrum" of an antibiotic. During the past ten or twelve years at least ten antibiotics of major clinical importance have been produced and merchandised on a commercial scale. Certain of these have become popularized as "broad-spectrum" antibiotics, and others have been referred to as having a "limited spectrum" of antibacterial activity. The connotations of these terms are somewhat misleading, since all the antibiotics affect only a limited number of kinds of microorganisms, and too little emphasis has been given to the desirability of effective and specific inhibition of a pathogenic microorganism.

Though many kinds of bacteria could be described as being uniformly sensitive, it was soon recognized that among certain species of bacteria different strains might vary greatly in their sensitivities to a particular antibiotic. Thus, by determining the effectiveness of different antibiotics under similar conditions of testing against a large group of strains from a single bacterial species, "antibiotic spectrums" also have been constructed for most of the kinds of microorganisms that commonly are pathogenic for human beings. These data indicate the relative potency in vitro of the antibiotics and the variability and range of the sensitivity of different strains of the same species for each antibiotic. If these conditions were static, there would be little need for continuing sensitivity tests, but because of the emergence of resistant strains, both the bacterial and the antibiotic spectrums have changed appreciably since the introduction and widespread clinical use of most of the antibiotics.

INDICATIONS FOR DETERMINING BACTERIAL SENSITIVITY

Determinative bacteriology is utilized to assist the physician in establishing a definitive etiology of infectious illnesses among his patients. The testing of bacterial strains for their sensitivity to different antibiotics is done to assist the physician in the selection of a therapeutic agent. The degree of success derived from either of these procedures depends upon the time in the illness and the care with which the collection of specimens for study is made, and upon sufficient familiarity on the part of the physician with the laboratory methods as well as the clinical illness to interpret the results meaningfully.

Bacterial cultures should be obtained preferably before or at the time of the initiation of treatment. Once antibiotics have been given, the

TABLE 17. *Spectrum of Antibiotic-Sensitive Bacterial Species as Determined by in Vitro Sensitivity Tests*

ANTIBIOTIC	BACTERIAL SPECIES AMONG WHICH DIFFERENT STRAINS ARE UNIFORMLY SENSITIVE	MAY BE SENSITIVE, BUT VARY CONSIDERABLY
Penicillin	Pneumococcus Hem. streptococcus, group A Diphtheria Gonococcus H. influenzae H. pertussis	Staphylococcus Hem. streptococcus, groups B, C and G Enterococcus Alpha streptococcus Gamma streptococcus Proteus
Streptomycin and Neomycin	Few, if any	Staphylococcus Coliform bacilli Aerobacter Klebsiella Pseudomonas Proteus H. influenzae H. pertussis
Bacitracin	Staphylococcus Hem. streptococcus Pneumococcus Diphtheria	Few, if any
Polymyxin B	Pseudomonas Coliform bacilli Aerobacter H. influenzae H. pertussis Salmonella Shigella	Few, if any
Chloramphenicol	Pneumococcus Hem. streptococcus Gonococcus Salmonella Shigella H. influenzae	Staphylococcus Coliform bacilli Aerobacter Klebsiella Proteus
Chlortetracycline, Oxytetracycline, Tetracycline	Pneumococcus Hem. streptococcus Gonococcus Clostridia H. influenzae H. pertussis	Staphylococcus Enterococcus Coliform bacilli Bacteroides
Erythromycin	Pneumococcus Hem. streptococcus	Staphylococcus

pre-existing flora of microorganisms in the patient becomes disturbed and a potentially pathogenic species that may be isolated subsequently cannot necessarily be assumed to be the primary cause of an infection. Furthermore, after treatment has been started it may be impossible to

isolate a pathogenic organism even though the patient has not been cured of the infection. Though the etiologic diagnosis of an infection often requires only the recognition of a few colonies of a pathogenic species, the strain must be isolated and propagated in pure culture to perform a bacterial sensitivity test; therefore additional time is required.

It is necessary for the physician to be familiar with the normal bacterial flora of the area from which a specimen for culture is taken, to know something of the common contaminants that often appear in such cultures, and to have a suspicion from the clinical findings as to what species of pathogenic microorganisms might be expected to cause the patient's symptoms. For example, it is desirable to determine the sensitivity of an alpha hemolytic streptococcus obtained from the blood of a patient with a heart murmur, whereas similar study of an alpha hemolytic streptococcus from a throat culture would usually be a waste of time. Also, the request by a physician for "routine" sensitivity tests or sensitivity test to "all the antibiotics" indicates insufficient understanding of the limitations and practical value of the procedure. In most instances it should be possible for the physician to select two or three antibiotics for study, based on the bacterial etiology of the infection and the clinical applicability for administration to the patient.

Some species of bacteria among which different strains vary greatly in their susceptibility to different antibiotics are shown in Table 17. In these instances the determination of the relative effectiveness of several antibiotics may be highly advantageous. Other species of bacteria are uniform in the sensitivity of different strains to a given antibiotic. In the latter case, sensitivity studies are uncalled for, except perhaps in a few research centers that are on the lookout for the development of resistant strains among these species.

In a few diseases it is nearly always desirable to have sensitivity studies performed. These are illnesses that require prolonged treatment to obtain a bacteriologic cure, such as bacterial endocarditis, osteomyelitis, intractable abscesses and tuberculosis. On the other hand, in a few chronic diseases such as brucellosis, tularemia and the typhoid carrier state, sensitivity tests may be misleading and, in addition, dangerous for the laboratory personnel to perform.

EVALUATION OF THE METHODS FOR DETERMINING SENSITIVITY

It behooves the physician to know something of the accuracy of the methods and the pitfalls involved in determining the minimal inhibitory concentration of an antibiotic in vitro. The minimal inhibitory concentration, or MIC, is the lowest concentration of an antibiotic that will inhibit the growth of a given strain of bacteria. As previously noted, there are many methods that may be used to observe the effects of

antibiotics upon bacteria in vitro, and the minimal inhibitory concentration for any one strain depends upon the conditions of the test. Three methods principally are used in laboratories for clinical bacteriology: (1) the medicated disk method, (2) the tube-dilution method, and (3) a tube-to-plate method.

Medicated Disk Method

In this method a medicated disk is applied to an agar surface that has been seeded previously with an unmeasured number of microorganisms. Two or three disks impregnated with different amounts of a dry salt of an antibiotic generally are used. The antibiotic is diluted by diffusion into the agar, and the degree of diffusion varies, depending upon the moisture, agar content, temperature and duration of contact. The presence or absence of a zone of inhibited growth surrounding the disk is used to indicate sensitivity or resistance to different amounts of the antibiotic. Usually no attempt is made to estimate the minimal inhibitory concentration, but sometimes the area of the clear zone is interpreted as an indication of the degree of sensitivity. This is not considered a reliable measurement, however, and the method is designed only to enable an estimation of whether the strain is sensitive, moderately resistant or very resistant to an antibiotic.[1] In some cases the results may be misleading. Some authors[3] have called attention to the "possible hazards associated with the use of the various disk and tablet methods for assaying the sensitivity of bacteria to various antibiotics in the clinical laboratory," and others[2] have shown the frequent lack of agreement between the disk method and other procedures for determining sensitivity in vitro. A good correlation between disk sensitivities and clinical response to treatment does not necessarily validate the method, since in the consideration of a large number of patients the majority will recover from infectious diseases and may respond to less than optimal therapy. To obtain the most accurate information about the sensitivity of bacteria from a severely ill patient, the disk method is not recommended. The technique might be useful, however, in screening some of the gram-negative bacilli for sensitivity to the tetracycline drugs, chloramphenicol and, if a dense inoculum is used, possibly streptomycin also.

Tube-Dilution Method

In the tube-dilution method accurate concentrations of the antibiotic are made in broth and then serially diluted. The volume and number of bacteria inoculated into the antibiotic broth also are known. Thus the minimal inhibitory concentration can be determined. In the usual

method the accuracy of the result can be considered to be from one half to twice the mean or true value. For example, a strain reported to be sensitive to 0.5 unit per milliliter may be inhibited at another time by 0.25 unit per milliliter or it may require 1 unit per milliliter to inhibit growth under the same conditions. Similarly, a strain that is found sensitive to 10 units per milliliter may be inhibited by 5 units per milliliter or require as much as 20 units per milliliter for inhibition. It is obvious that the therapeutic implications of the difference between 5 and 20 units per milliliter are important in obtaining appropriate serum concentrations of some of the antibiotics, and occasionally the difference between 0.25 and 1 unit per milliliter might be significant in the body fluids.

Tube-to-Plate Method

The tube-to-plate method of testing sensitivity is an extension of the tube-dilution method and is perhaps the most informative and reliable of the three methods discussed for direct application to the therapeutic requirements for the patient. Both the rate and time of bacterial killing by various concentrations of the antibiotic can be observed by streaking the culture or making poured agar plate counts of the number of viable bacteria at different times during the incubation period of the bacteria with the antibiotic.

CRITERIA OF BACTERIAL INHIBITION

Different criteria of bacterial inhibition are applied in these methods. In the first two the measure of inhibition is the suppression of grossly visible growth for eighteen to twenty-four hours. This does not necessarily mean that the bacteria have been killed or even 100 per cent inhibited, whereas in the tube-to-plate method the bactericidal effect of the antibiotic is measured.

The number of microorganisms in the inoculum tested for sensitivity also is of great importance when the antibiotic in question is penicillin or streptomycin. It is not uncommon for a clinician to be consulted about a penicillin-sensitive staphylococcus that has not responded to treatment. As illustrated in Table 18, a strain of staphylococcus that produces penicillinase will appear sensitive to penicillin when the test inoculum contains a small number of microorganisms (100 to 10,000 per milliliter), but 250 units per milliliter or more may not inhibit the same strain when a large inoculum (100,000 organisms or more) is used for the test. This variation also is frequently the basis for shifts in sensitivity to penicillin of cultures obtained during treatment which appear to be valid, whereas in reality there is no change at all if a

uniform inoculum is used for the test. The importance of the size of the inoculum has been observed for species of bacteria other than staphylococcus when they are tested for sensitivity to penicillin or streptomycin.[2, 4]

A number of methods for studying combinations of antibiotics have been proposed, but to date perhaps the most interpretable one is the adaptation of the tube-to-plate method which indicates the rate and completeness of bacterial killing with different concentrations of the

TABLE 18. *Effect of Size of Inoculum upon in Vitro Sensitivity of a Penicillinase-Producing Strain of Staphylococcus Aureus*

DATE ISOLATED	MINIMAL INHIBITORY CONCENTRATION OF PENICILLIN, UNITS/m			
	Number of Microorganisms per ml. in the inoculum tested			
	1 Million	100,000	10,000	1000
12/5	> 250	> 250	125	3.9
12/7	> 250	250	31.3	0.49
12/8	> 250	> 250	31.3	1.95
12/9	> 250	125	15.6	0.98
12/10	> 250	250	62.5	1.95

antibiotics in combination. Sensitivity tests using sulfonamides are extremely unreliable.

INTERPRETATION AND SIGNIFICANCE OF SENSITIVITY TESTS IN VIVO

The sensitivity of bacteria to antibiotics is an in vitro characteristic of the strain. It is not a system of identifying bacteria or an absolute index of the therapeutic efficacy of an antibiotic in vivo. Young physicians who received their entire training in the period when many antibiotics were available have been heard to say, regarding the results of a bacteriologic culture, "I don't care what it is, what do the sensitivities show?" The science of antibiotic sensitivity testing is not so accurate or important that we should allow it to encroach upon proper determinative bacteriology, either in our clinical thinking or by occupying too much of the bacteriologists' time.

In certain instances the determination of bacterial sensitivity in vitro has been of invaluable aid in the selection of a therapeutic agent and in establishing the dose required. Even though the antibiotic sensitivity in vitro cannot be quantitatively assumed to apply in vivo, the correlation with response to therapy of experimental infections in animals is close if one obtains the in vitro inhibitory concentration at the site of the infection for a sufficient time. In order to accomplish this, a rule of thumb has been to give a dose sufficient to obtain a serum concentration four to five times the sensitivity in vitro of the infecting organism. In cases in which there is a barrier to the transport or con-

centration of the antibiotic at the site of the infection, as in meningitis, one cannot rely upon the sensitivity of the strain in vitro with regard to treatment except as the drug reaches the infected region. Some cases of pneumococcal meningitis and a few reported cases of meningitis caused by *Listeria monocytogenes* are examples of fatal infections with penicillin-sensitive organisms that were not cured with penicillin treatment.

There are other paradoxical situations in which microorganisms sensitive to an antibiotic in vitro do not behave similarly in vivo: for example, failure to eliminate typhoid bacilli in the chronic carrier state by the use of chloramphenicol, to which the organisms are sensitive in the test tube, and the poor clinical effect of the tetracycline drugs on clinical tuberculosis. On the other hand, chlortetracycline is effective in the treatment of brucellosis, even though the inhibition in vitro is poor.

The duration of treatment, of course, cannot be judged by the degree of sensitivity in vitro or lack of it. It is better related to the rate of proliferation of the microorganisms or the age and nature of the pathologic lesion. Subacute bacterial endocarditis may be cited as an example in which bacterial strains that are very sensitive to penicillin in vitro may persist in the endocardial vegetations for weeks despite high plasma concentrations of penicillin.

The plasma concentration of an antibiotic is not comparable to the inhibitory concentration for a bacterial strain except when the same method has been used in both determinations, and then the errors in each determination must be considered. The plasma levels that have been observed from the commonly administered doses of some antibiotics have set the standard of a sensitivity strain as one that is inhibited in vitro by 1 unit or microgram per milliliter of the antibiotic. Moderate sensitivity may be assumed for strains inhibited by less than 10 units or micrograms per milliliter, but strains not inhibited at this concentration in vitro must be assumed to be resistant to eradication in vivo after ordinary doses of most of the antibiotics.

BACTERIAL RESISTANCE TO ANTIBIOTICS

The occurrence of resistant strains of bacteria is perhaps the principal reason for the need to continue performing sensitivity tests in vitro. However, bacterial resistance to antibiotics is of at least two types: (1) species of microorganisms that have natural resistance common to nearly all the individual strains within a species, and (2) species that are naturally sensitive to an antibiotic, but from which individual colonies or mutant cells have given rise to strains resistant to one or more antibiotics. Species of the former type are sometimes referred to as a hiatus in the activity of the antibiotic. Among the latter group

TABLE 19. *Spectrum of Antibiotic Hiatuses for Different Bacterial Species as Determined by in Vitro Sensitivity Tests*

BACTERIAL SPECIES	DEFINITE HIATUS	RELATIVE HIATUS
Pneumococcus	Polymyxin B	Streptomycin
Hem. streptococcus	Streptomycin Polymyxin B	Sulfonamides
(Group A)	Streptomycin	Penicillin
(Groups B, C, G)	Polymyxin	Sulfonamides
Enterococcus, alpha and gamma streptococcus	Streptomycin Bacitracin Polymyxin B	Penicillin Erythromycin Tetracyclines
Staphylococcus	Polymyxin B Sulfonamides	Sulfonamides Penicillin Streptomycin Tetracyclines Erythromycin
Diphtheria	Polymyxin Streptomycin	
Gonococcus	Polymyxin Streptomycin	Sulfonamides
Coliform bacilli	Penicillin Bacitracin Erythromycin	Streptomycin Chloramphenicol Tetracycline
Aerobacter and Klebsiella	Penicillin Bacitracin Erythromycin	Streptomycin Chloramphenicol Tetracyclines
Proteus	Tetracyclines Erythromycin Bacitracin Polymyxin	Penicillin Streptomycin Chloramphenicol
Pseudomonas	Penicillin Erythromycin Tetracyclines Chloramphenicol Bacitracin	Streptomycin Sulfonamides
Salmonella	Bacitracin Streptomycin Erythromycin Sulfonamides	Penicillin } Except Tetracyclines } typhosa
Shigella	Penicillin Bacitracin Erythromycin Streptomycin Tetracyclines	
Hemophilus	Bacitracin Erythromycin	Penicillin Streptomycin Sulfonamides
Common yeasts (Candida)	All	

there are some species from which the emergence of resistant strains has been so complete that they also comprise a significant hiatus in the activity of some of the antibiotics. Knowledge of the major hiatuses of an antibiotic is helpful in selecting an agent for treatment before the results of the sensitivity test are available, and also in identifying the antibiotics that it will be most profitable to study in the laboratory. A spectrum of "antibiotic hiatuses" for different bacterial species as determined by sensitivity tests in vitro is presented in Table 19. It is generally unnecessary to determine the sensitivity in vitro of individual strains among these species when a definite hiatus has been recognized; and if such a strain is reported to be sensitive, it is reasonable to request that the results be checked.

The emergence of resistant strains varies in different areas and according to the extent to which an antibiotic is used. Thus some relative hiatuses in antibiotic activity have been indicated that will be different in different hospitals and in patients in the hospital and at home. However, a considerable number of resistant strains may be expected among the species when a relative hiatus has been indicated.

Some bacterial species are difficult to propagate, or the conditions for bacterial growth are not suitable for measuring antibiotic activity, and too few data are available for a proper evaluation. Among this group are some of the obligate anaerobic organisms such as bacteroides and clostridia. Others are streptobacillus, listeria, leptospira and pleuropneumonia-like organisms.

SUMMARY AND DISCUSSION

Tests in vitro for the effects of an antibiotic upon bacteria can be adapted to yield a number of types of specific information; however, in clinical practice the sensitivity test is used to indicate the minimal concentration of an antibiotic that will suppress the growth of or kill a bacterial strain.

From the application of such tests to a large variety of species of microorganisms the "bacterial spectrums" of the antibiotics have been determined. From the relative effect of different antibiotics upon many strains of a single species of bacteria, "antibiotic spectrums" also have been constructed for different kinds of bacteria. In certain bacterial species that have been found to have wide differences in the sensitivity of individual strains the determination of the minimal inhibitory concentration of an antibiotic is desirable, but it is unnecessary for other species that have been found to be uniformly sensitive or resistant.

In the opinion of the author, "routine" sensitivity testing is overdone and its therapeutic implications commonly are abused. The experience with antibiotics, both in the treatment of patients and in the laboratory,

has accumulated into patterns with some predictability, and there is little need for routine repetition.

Three principal methods for measuring the sensitivity in vitro of bacteria to antibiotics are used in clinical laboratories. These are the medicated disk, serial tube-dilution, and tube-to-plate methods. The results obtained show considerable variation according to the method used, and sometimes the results of sensitivity tests are misleading so far as their application in vivo is concerned. The characteristics of the clinical infection and the status of the patient still may be more important aids in the choice of a therapeutic agent and the dose required than is the sensitivity test, if the bacterial etiology has been identified. In those illnesses in which a sensitivity test is needed, a tube-dilution or tube-to-plate method is most desirable.

Plasma levels of an antibiotic may not be comparable to the inhibitory concentration in vitro for a pathogenic microorganism. Only strains that are inhibited in vitro by 1 unit or microgram per milliliter or less of an antibiotic can be assumed to be sensitive in vivo to the administration of ordinary doses, and then only if the inhibitory concentration is obtained at the site of the infection for a sufficient length of time.

REFERENCES

1. Eisenberg, G. M., and Wagner, B. M.: Bacterial Susceptibility to Antibiotics. II. Determination by Means of a Standardized Routine Procedure Having Clinical Significance. Am. J. M. Sc., 223:600, 1952.
2. Jackson, G. G., and Finland, M.: Comparison of Methods for Determining Sensitivity of Bacteria to Antibiotics *in Vitro*. Arch. Int. Med., 88:446, 1951.
3. May, J. R., and Morley, C. W.: The Routine Testing of the Sensitivity of Bacteria to Antibiotics. Lancet, *1*:636, 1952.
4. Rash, C., and Parker, R. F.: Action of Penicillin on Streptococci: Enhancement of Sensitivity *in Vivo?* Science, *116*:16, 1952.

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FLUID AND ELECTROLYTE THERAPY

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The objective of parenteral fluid therapy is the orderly restitution of the state of well-being by the reversal of the functional impairments resulting from distortions of the body fluids.

Insufficient knowledge of body fluid physiology, lack of practical methods for accurately determining the composition of the fluids and, particularly, our inability to assess the functioning of the regulatory mechanisms initially and during treatment preclude precise management. Recognition of these limitations is helpful because it emphasizes the need for thoughtful consideration of each clinical situation, the necessity for new and changing concepts, and because it decreases the urge for an unvarying "best method" of treatment. Disturbances in the composition of the body fluids are usually complex and frequently highly individualized. Therefore undue "simplification" and "routines" of therapy may be understandably of limited value in the management of a particular patient.

Nevertheless, guiding principles are necessary. The ones discussed here, if combined with thoughtful evaluation of the patient and consideration of what is known about body fluid physiology, will generally lead to satisfactory management.

DIAGNOSIS

Appropriate treatment depends upon understanding what is the matter with the patient: (1) the basic disease producing the disturbance, (2) the alterations in the composition of the body fluids, and

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TABLE 20. *Symptoms and Signs Often Observed in the Designated Disturbances of the Body Fluids*

	ISOTONIC REDUCTION IN FLUID VOLUME	HYPO-OSMOLARITY	HYPEROSMOLARITY	POTASSIUM DEFICIT WITHOUT DECREASED FLUID VOLUME
Nervous system:				
Mental state	Fatigue Apathy Somnolence Disinterest in surroundings	Headache Coma Convulsions Increased cerebrospinal fluid pressure	Restlessness Delirium Maniacal behavior	Weakness Lethargy Depression Apprehension Confusion (Especially symptoms persisting after correction of volume and tonicity derangements, alkalosis resistant to treatment)
Reflexes	Hypoactive	Hyperactive		
Cardiovascular system:	Tachycardia	Bradycardia	Tachycardia	Tachycardia
Heart	Decreased heart sounds Decreased A2			Poor heart sounds Enlargement Appearance of murmurs Extrasystoles
Blood pressure	Falling blood pressure	Hypertension to low blood pressure		Widening pulse pressure Hypotension
Pulse	Soft, rapid, thin			Collapsing pulse
Veins	Slow filling of veins			Elevated venous pressure Hepatic enlargement
Respiration		Slow Moist rales occasionally	Hyperpnea Sudden respiratory arrest	Dyspnea Shallow, gasping respirations Cyanosis

Gastrointestinal tract	Anorexia	"Coffee-ground" vomiting, water diarrhea No thirst	Marked thirst	Vomiting, abdominal distention, paralytic ileus
Tissues:				
Mucous membranes	Moist	Increased salivation	Dry, sticky mouth	Muscular weakness, flaccid paralysis; hypotonic, flabby muscles
Muscles	Pasty muscles			
Skin	Weak muscles	Muscle cramps and twitches	Fair skin turgor	May be peripheral edema
	Cool, pallid skin with loss of turgor	Skin flabby with loss of turgor	Flushed, warm skin	
Temperature	Hypothermia	Hypothermia	Hyperthermia	

(3) any impairment in the mechanisms regulating the composition of the body fluids. Therefore the initial step in treatment is to make a detailed working diagnosis of the body fluid derangement.

The physician has four aids which will assist him in visualizing the changes which have taken place in the cellular and extracellular compartments with respect to the major constituents—water, sodium, potassium, chloride, bicarbonate and phosphate, each being considered separately: (1) knowledge, derived from other studies, of the usual changes produced by the disease with which his patient is ill; (2) the patient's symptoms and his history of intake and abnormal losses of water and electrolytes, and weight loss; (3) those features of the physical examination indicative of changes in the content of body water, of alteration in the tonicity of the body fluids and of distortion in the ionic composition of the fluids; (4) appropriately interpreted laboratory measurements, if accurate and quickly available.

The clinical symptoms and signs are common to a variety of disturbances. However, if their probable significance, as well as their limitations, is kept in mind, the relevant symptoms and signs usually will lead to a useful definition of the body fluid disturbance. Table 20 depicts the main features observed in patients suffering from more or less "pure" types of the four derangements listed. Frequently only a few of the symptoms and signs listed in the table will be found in a given patient. In most clinical situations there will be a mixture of these symptoms and signs, because, initially at least, most patients will have combinations of reduction in fluid volume, alteration in tonicity, and may or, more likely, may not exhibit evidence of potassium deficiency. More nearly "pure" types of change in tonicity and potassium deficit are apt to be seen after initial hydration. Therefore, it is in planning and controlling continued parenteral fluid therapy that these physical signs are most useful.

In potassium deficiency perhaps no symptoms will be present, in some cases only evidence of dysfunction of smooth muscle, or in others cardiovascular disturbances alone may be found. Serious dysfunction may occur rapidly in certain conditions, such as the recovery phase of diabetic acidosis or after operations. Clearly treatment with potassium should be dictated by *need for it* and not by any fixed rules, such as so many hours after initial hydration, and the like.

Lastly, the same four aids—knowledge of similar patients, the history, the physical examination, and selected laboratory measurements—help one estimate any disturbance in the functioning of the osmoreceptor-hypothalamic-posterior pituitary system, an excess or deficit of "salt-retaining" hormones or possible renal dysfunction. Pain, trauma, infections, operations, circulatory impairment, anoxia and certain drugs may affect one, two or all three of these regulatory mechanisms, thereby necessitating some modification of the therapeutic plan.

GENERAL PRINCIPLES

1. *Immediate Therapy of Decreased Effective Circulation,* "shock," if present or strongly suspected. Rapid intravenous partial replacement of the extracellular fluid deficit is the first step, 20 to 30 ml. per kilogram of body weight. When *hypotonicity* of the body fluids is suspected, an isotonic electrolyte solution is chosen; whereas if *hypertonicity* is suspected, the initial fluid for therapy would be a mixture of equal parts of 5 per cent glucose in water and isotonic electrolyte solution. A transfusion of whole blood immediately after this first step renders it much more effective because of the space-occupying properties, the oxygen- and carbon dioxide-carrying functions and buffer value of whole blood. Plasma and human serum albumin are second best substitutes for whole blood at this stage. It is probable that 6 per cent dextran or 3.5 per cent polyvinylpyrrolidone would be as effective as plasma.

2. *Replacement of Much of the Pre-existing Deficits* of water and electrolytes as estimated in the detailed diagnosis. It is noteworthy that this requirement for electrolyte solution is needed only once and that there are limitations as to both the amount and the rate of administration of predominantly intracellular ions. Body weight is the best basis for calculating the pre-existing deficits, somewhere between 50 and 80 ml. of an appropriate electrolyte solution per kilogram of body weight.

3. *Provision of Sufficient Water* to meet the obligatory water expenditure from the lungs and skin and for urine formation. This provision of expendable water, which is proportional to calories burned, must be met each day and cannot be safely supplied as isotonic electrolyte solution. One to 1.5 ml. of glucose in water per calorie burned per day is the usual range of this obligatory requirement.

4. *Measures to Overcome Abnormal Metabolic Reactions,* to reduce abnormal losses and to return the patient to an oral intake of natural foods as soon as clinically feasible, e.g., relief from "stress" by chemotherapeutic, surgical or other measures, treatment of endocrine deficits by hormones, and giving sufficient carbohydrate to eliminate ketosis, diminish nitrogen loss and partially conserve extracellular electrolytes.

5. *Check to Prevent Producing Disturbances* as a result of treatment. Is the proper *type* of solution being used, is the *amount* inadequate or excessive, is the *route* appropriate, and what measures are taken so that the *rate* of administration is neither too rapid nor too slow?

6. *Coverage of Contemporary Losses* by an appropriate electrolyte solution in amounts proportional to the daily abnormal losses is necessary, usually in decreasing amounts, until such losses cease.

7. *The Application of Adequate Measures to Follow the Progress of the Patient.* This is perhaps the principle most often neglected. The measures in more or less order of their usefulness are as follows:

(*a*) accurate measurements of body weight; (*b*) a tabulation of the water and each electrolyte that the patient actually received during the previous twelve or twenty-four hours expressed in terms of some unit system, such as milliliters of water or milliequivalents of each electrolyte per kilogram of body weight (per square meter of body surface, or per 100 calories metabolized); (*c*) examination of the patient, especially with reference to mental status, reflexes, the cardiovascular system, respirations, gastrointestinal tract, mucous membranes, muscles and skin; (*d*) estimation of the type and amount of abnormal losses and evidence of disturbance in the regulatory mechanisms; (*e*) a few selected laboratory measurements, particularly if the course of the patient is unsatisfactory; (*f*) serial electrocardiographic tracings, especially when potassium therapy is being used.

SOLUTIONS FOR PARENTERAL THERAPY

A large variety of electrolyte and carbohydrate solutions are available commercially or may be easily made up as needed by diluting sterile concentrated solutions of various salts with distilled water or glucose in water. Hence only prototypes of various solutions will be shown in Table 21.

A. Solutions furnishing expendable water (and hypotonic multiple electrolyte solutions) should be given intravenously to avoid the temporary withdrawal of body electrolytes which is produced by subcutaneous glucose in water. Thiamine and ascorbic acid may be added to the glucose solutions.

C. Solutions containing potassium in a concentration of 20 to 50 milliequivalents per liter play a vital role in preventing symptoms in states of potassium deficiency. Potassium intoxication can be prevented (1) if care is taken not to use potassium solutions when adrenal insufficiency, severe renal impairment or marked oliguria is present or seriously suspected; (2) if one gives such solutions only when the type, severity and duration of the illness make it likely that a deficit of potassium exists; (3) no more than 3 to 4 milliequivalents of potassium per kilogram of body weight are given initially over a minimum period of six hours. (Larger amounts may be needed to control symptoms and obviously should be given under those circumstances.) The safest route for the parenteral administration of Darrow's solution or the KCl-NaCl solution is by hypodermoclysis. These solutions may be given intravenously with safety if they are diluted with the glucose in water being used to provide expendable water. Serial electrocardiograms will reveal important elevations of the serum potassium level. Special precautions to prevent too rapid a rate of administration must be taken when solutions containing phosphate, ammonium or calcium ions are

TABLE 21. *Fluids Used in Parenteral Fluid Therapy*

SOLUTION	COMPOSITION						ROUTE		RANGE OF DAILY AMOUNT		CHIEF USE
	Na mEq.	K mEq.	Cl mEq.	PO_4 mEq.	NH_4 mEq.	CHO Gm.	IV.	S.C.	Ml. per kg.	Ml. per cal.	
A. **Glucose in water**	0	0	0	0	0	50–100	Yes	Some cases		1–1.5	Provide expendable water, carbohydrate and calories
B. **0.9% Saline**	154		154				Yes	Yes	20–40		Alkalosis, initial therapy
Hartmann's											
Ringer's lactate	131	4	107				Yes	Yes	20–40		Acidosis, initial therapy
Saline-Lactate											
(2 pts: 1 pt. $\frac{M}{6}$)	158		103				Yes	Yes	20–40		Acidosis, initial therapy
C. **Darrow's**	122	35	103				With care	Yes	50–80		Acidosis, replacement and maintenance therapy
KCl-NaCl (2.7–6 gm. per liter)	102	36	138				With care	Yes	50–80		Alkalosis, replacement and maintenance therapy
D. **Butler-Talbot**	55	23	45	12		25–100	Yes	—		1–2	Acidosis, esp. diabetic acidosis replacement and maintenance therapy
E. **NH_4-Na-K-Cl**	60	20	150		70		With care		Equal to loss		For volume-for-volume replacement of upper G.I. secretions removed by suctioning

administered. The evidence for the use of intravenous solutions containing small amounts of calcium or magnesium is not too clear to date. Be sure that calcium salts are not added to solutions containing phosphate.

Although the anion lactate has been mentioned in describing the solutions, there is evidence that solutions in which acetate is used in place of lactate are more efficacious and probably should be used when available.

In many instances of body fluid disturbances maintenance amounts of water, carbohydrates, sodium, chloride, potassium, phosphate and, if desired, calcium and magnesium can be taken orally after the initial replacement of pre-existing deficits. Such therapy shortens the period of parenteral fluid administration, simplifies the patient's management, and allows increased amounts of intracellular ions to be given safely.

APPLICATION

Case History Illustrating Diagnosis and the Principles of Management

A., a 6 weeks old male infant, weighed 2.6 kilograms at birth and did well for 5 weeks on an artificial feeding and weighed 3.3 kilograms at that time.

Six days before admission he had diarrhea consisting of 6 to 8 loose, large, watery stools each day until admission. For the first 2 days he seemed alert and took his feedings as usual. For the following 2 days he gradually became listless and refused more and more of his feedings, although he eagerly took several ounces of water each day. The day before admission he vomited twice, refused even water and became alternately lethargic and restless.

On admission he weighed 2.81 kilograms. He appeared gravely ill and was stuporous, but had periods of irrational hyperactivity. Examination showed no evidence of respiratory tract or otitic infection. There were hypoactive reflexes, tachycardia, heart sounds of good quality, moderately full pulse and fair peripheral circulation. Moderate hyperpnea was present, the lungs were clear, and the abdomen appeared normal except for hyperperistalsis. The mucous membranes were markedly dry, the fontanel and eyes sunken, and the skin turgor was fair. Over the body the skin was warm, but it was cold and mottled over the extremities. The rectal temperature was 104° F.

Blood drawn before therapy and reported later showed: Carbon dioxide content, 9 milliequivalents per liter; chloride, 126 milliequivalents per liter. Urine passed soon after starting treatment had a *p*H of 5.9, specific gravity of 1.016, 1+ albumin and a rare red blood cell.

Interpretation. From the history of weight loss, decreasing intake of food and eventually even water, the abnormal losses by diarrhea and finally vomiting, and from the age of the patient one could surmise that there would be an extensive reduction in body water and electrolyte, with a relatively greater loss of water than electrolyte likely. The mental status suggests grave fluid reduction plus hyperosmolarity of the body fluids. This impression seems to be borne out by the relatively good circulation and skin turgor in the face of such marked dehydration plus the dry mucous membranes and the elevated temperature. The respirations and carbon dioxide content are consistent

with the expected metabolic acidosis. The sum of the carbon dioxide content and the chloride concentration probably indicates hypertonicity. Some impairment of renal function is suggested by the fact that the urine is not more acid in the face of such a marked systemic acidosis and that the concentration of the urine is not higher.

Management. For the first 18 to 24 hours 5 steps are required: (1) Immediate treatment of "shock" by 30 ml. per kilogram of body weight of a mixture of equal parts of 5 per cent glucose in water and saline-lactate intravenously followed by 20 ml. per kilogram of whole blood. (Had there been *hypotonic* instead of hypertonic dehydration, 30 ml. per kilogram of isotonic saline-lactate would have been used initially.) (2) Partial replacement of pre-existing deficits by 80 ml. per kilogram of body weight of Darrow's solution subcutaneously over at least a 6 hour period. (3) Provision of expendable water by 450 ml. of 5 per cent glucose in water plus thiamine and ascorbic acid intravenously, calculated as 1.5 ml. per calorie expended. (4) Ancillary treatment: vitamin K. (Antibiotics if desired, although their influence on diarrheal disease is dubious.) (5) Check types, amounts, routes and rates of administration of all fluids.

Subsequent Course. At the end of 20 hours the patient was alert, had good circulation, moderate hyperpnea, no vomiting, normal temperature and had had 5 watery stools. Then, inadvisedly, he received 600 ml. of only 5 per cent glucose in water orally during the next 16 hours. In this period he had only 2 large loose stools. Then he became distended, vomited twice and had a generalized convulsion. Examination revealed a comatose infant with a tense fontanel, hyperactive reflexes, heart sounds of poor quality, rapid Corrigan type of pulse, distended silent abdomen with a slightly enlarged liver, slow shallow respirations, moist mucous membranes, flabby hypotonic muscles and a body temperature of 96° F. His weight was 3.42 kilograms.

Blood drawn at this time had a serum sodium concentration of 124 milliequivalents per liter, serum potassium of 3.1 milliequivalents per liter, serum calcium of 8.9 mg. per 100 ml., and a blood sugar of 140 mg. per 100 ml.

Interpretation. From the change in body weight, the hypothermia, the type and amount of intake and losses and the convulsion one might suspect *hypo*tonicity of the body fluids as the cause of the convulsion. Some of the physical findings confirmed this impression, but the cardiac, gastrointestinal and skeletal muscle findings also suggested that clinically important potassium deficit was present. The laboratory data and an electrocardiographic tracing confirmed both these impressions as well as ruled out hypocalcemia or hypoglycemia as a cause for the convulsion.

Before the laboratory reports were back, 1 gm. of potassium chloride in 50 ml. of water was given by mouth, and then 30 ml. of a mixture of equal parts of 1.8 per cent saline and third-molar sodium lactate solution were given slowly intravenously. No additional fluids were given for 12 hours. Thereafter the patient made a good recovery.

Comment: The plan for managing this patient after the first twenty-four hours of parental fluid therapy should have consisted of four steps: (1) Evaluation of the progress of the patient, particularly with reference to (*a*) change in body weight, (*b*) the amounts of water and electrolyte actually given, (*c*) examination of the patient, (*d*) estimation of abnormal losses and disturbances of the regulatory mechanisms, (*e*) review of electrocardiographic and any laboratory data. Does the patient require parenteral fluid therapy, or can the needed electrolyte and glucose in water solutions be mixed and given orally?

2. Provision for expendable water: 1 to 1.5 ml. per calorie burned of 5 to 10 per cent glucose in water.

3. Replacement of continuing losses: 10 to 40 ml. per kilogram of body weight of Darrow's solution.

4. Ancillary therapy as needed.

This case presentation illustrates that laboratory measurements are not usually required for planning the first day's therapy and can often be dispensed with in following the progress of the patient, although, as in this case, they were used to confirm objectively the tentative diagnosis arrived at by clinical measures.

SUMMARY

The impairment of well-being resulting from distortions of the body fluid composition can generally be relieved despite the limitations in our present knowledge and in our inability to make an objective diagnosis.

The importance of a detailed working diagnosis is emphasized, and the steps useful in making it are pointed out.

The seven general principles of management are the following: (1) prompt treatment of "shock"; (2) replacement of much of the pre-existing deficits; (3) provision of expendable water; (4) measures to overcome abnormal metabolic reactions; (5) prevention of disturbances due to the therapy; (6) coverage of contemporary losses; (7) adequate measures to follow the course of the patient.

REFERENCES

1. Butler, A. M.: Diabetic Coma. New England J. Med., *243*:648, 1950.
2. Chung, A. W., and Holt, L. E., Jr.: Place of Oral Feeding in Infantile Diarrhea. Pediatrics, *5*:421, 1950.
3. Darrow, D. C., and Pratt, E. L.: Fluid Therapy, Relation to Tissue Composition and the Expenditure of Water and Electrolyte. J.A.M.A., *143*:365, 432, 1950.
4. Darrow, D. C., and Pratt, E. L.: Retention of Water and Electrolyte during Recovery in a Patient with Diabetic Acidosis. J. Pediat., *41*:688, 1952.
5. Eliel, L. P., et al.: Postoperative Potassium Deficit and Metabolic Alkalosis. New England J. Med., *243*:471, 518, 1950.
6. Flett, J., Jr., Pratt, E. L., and Darrow, D. C.: Methods Used in Treatment of Diarrhea with Potassium and Sodium Salts. Pediatrics, *4*:604, 1949.
7. Gamble, J. L.: Chemical Anatomy, Physiology and Pathology of Extracellular Fluid. 5th ed. Cambridge, Harvard University Press, 1947.
8. Moore, F. D.: Adaption of Supportive Treatment to Needs of the Surgical Patient. J.A.M.A., *141*:646, 1949.
9. Moore, F. D.: The Low Sodium Syndromes of Surgery, an Outline for Practical Management. J.A.M.A., *154*:379, 1954.
10. Moyer, C. A.: Fluid Balance. Chicago, Year Book Publishers, Inc., 1952.

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